THE EFFECTS OF CYANIDES ON AQUATIC ORGANISMS WITH EMPHASIS UPON FRESHWATER FISHES

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ANNOUNCEMENT
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PUBLICATION

THE EFFECTS OF CYANIDES ON AQUATIC ORGANISMS WITH EMPHASIS UPON FRESHWATER FISHES

This document begins with a survey of the chemistry and the environmental dynamics of cyanides in aquatic ecosystems. It then deals in detail with the toxicological effects of cyanides on aquatic organisms and concludes with an analysis of the overall significance of laboratory studies.

While the ubiquitous nature of cyanide-containing compounds in terrestrial and aquatic ecosystems is attributable to both natural and man-made sources, the industrial age has brought on increases in levels to the point where cyanides are considered among the major pollutants in receiving waters.

The primary toxic form of cyanide in the aquatic environment is free cyanide, composed of the equilibrium summation of molecular HCN and the cyanide ion. In natural waters with a pH of 8.5 or lower, most of the free cyanide is in the form of molecular HCN. The toxicity of complex cyanides is usually related to the degree of release of cyanide ions to the solution, which then enter into an equilibrium with molecular HCN.

The toxicity of free cyanide at the lethal and sublethal levels of exposure has been extensively studied in the laboratory, using freshwater fish that had not been previously exposed to cyanide-containing compounds. In addition, fixed concentrations of toxicant were usually used for the duration of the tests. Although such test conditions are not found in the natural environment, the investigations indicated the toxicologically effective range of free cyanide concentrations and permitted a qualitative description of specific effects in fish.

With the exception of information concerning the effects of molecular HCN on freshwater fish, the current scientific criteria are insufficient to provide a thorough assessment of cyanide compounds in the Canadian aquatic environment. Numerous pieces of scientific information are lacking and recommendations for research are included in this document.

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NOUVELLE

PUBLICATION

EFFETS DES CYANURES SUR LES ORGANISMES AQUATIQUES, NOTAMMENT SUR LES POISSONS D'EAU DOUCE

Ce document passe en revue la chimie et la dynamique des cyanures dans les systèmes aquatiques, puis traite des effets toxicologiques de ces composés sur les organismes aquatiques et, enfin, les auteurs analysent la signification générale des études au laboratoire.

Les composés contenant des cyanures se trouvent partout dans les écosystèmes terrestres et aquatiques et proviennent des sources naturelles et anthropiques. Cependant, avec l'ère industrielle on remarque que les concentrations des cyanures augmentent au point où ils sont considérés parmi les polluants les plus importants dans les eaux réceptrices.

Le cyanure libre, composé de HCN moléculaire et d'ion cyanure à l'état d'équilibre, est la forme toxique la plus importante dans l'environnement aquatique. Dans les eaux naturelles (pH 8.5 au maximum), la plupart se trouve en HCN moléculaire. Normalement, la toxicité des cyanures complexes est associée à la proportion d'ions cyanures libérés dans la solution puis se mettant en équilibre avec le HCN moléculaire.

On a étudié en détail au laboratoire la toxicité du cyanure libre aux concentrations létales et sublétales au moyen de poissons d'eau douce qui n'avaient jamais été exposés aux cyanures. En outre, on a utilisé des concentrations fixes d'agents toxiques pendant les essais biologiques. Bien que de telles conditions ne soient pas observées dans la nature, les recherches ont permis de définir la plage toxique des concentrations de cyanures libres et de décrire de façon qualitative les effets spécifiques chez les poissons.

Mis à part les renseignements concernant les effets du HCN moléculaire sur les poissons d'eau douce, les critères scientifiques actuelles ne suffisent pas à permettre une évaluation complète des composés de cyanures dans l'environnement. Il manque beaucoup de données scientifiques et les auteurs proposent des recommandations de recherches pour combler ces lacunes.

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FOR
ENVIRONMENTAL QUALITY

THE EFFECTS OF CYANIDES ON AQUATIC ORGANISMS WITH EMPHASIS UPON FRESHWATER FISHES

Prepared for the Subcommittee on Water

by

G. Leduc, R.C. Pierce and I.R. McCracken

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All documents published by the Associate Committee are published in both French and English.

PANEL ON CYANIDES IN THE AQUATIC ENVIRONMENT[α]

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SUMMARY

The ubiquitous nature of cyanide-containing compounds has been well established; such widespread occurrence in terrestrial and aquatic ecosystems is attributable to both natural and man-made sources. With the advent of the industrial age, cyanides were considered among the major pollutants of receiving waters. As a result, numerous investigations on their removal have been carried out and various measures were taken to control their release via waterborne effluents.

In the ensuing years, the fate and effects of cyanides released from industrial sources were perceived mostly through investigations into severe cases of water pollution that produced acutely toxic conditions. Concern has been expressed as to whether these investigations, although significant in themselves, were adequate in providing the scientific criteria necessary for describing the physicochemical behavior and related toxicological and ecological effects of cyanide-containing compounds in natural waters. In order to establish sound scientific criteria for cyanide compounds in the aquatic environment, several critical questions must be addressed. These include:

(i) What are the actual forms of cyanide compounds in natural waters?

(ii) What are the dynamics of these forms in dilute aqueous systems and what modifying factors play a critical role in governing such dynamics?

(iii) What are the toxicological and ecological implications of the presence of cyanide compounds in natural waters, particularly at the chronic and sublethal levels of exposure?

With the exception of information concerning the effects of molecular HCN on freshwater fish, the scientific criteria are insufficient to provide anything more than a cursory assessment of cyanide compounds in the Canadian aquatic environment. Numerous pieces of scientific information are lacking and recommendations for research are included later in this document. A brief overview, highlighting the currently available information, follows.

1.0 CHEMISTRY (see Chapter 2.0)

Cyanides comprise a diverse group of inorganic and organic compounds characterized by the group -C≡N. Free cyanide refers to the summation of molecular HCN and the cyanide ion, CN^-, in aqueous solutions, regardless of their sources in water. Simple cyanide refers to a cyanide compound, such as sodium cyanide, that readily dissociates in water to a cation and the cyanide ion. Complex cyanide is that

cyanide which is incorporated into a complex or complexes; such a complex may dissociate in water to a cation and an anion containing cyanide. The anion, termed a complex cyanide ion, may further dissociate to the cyanide ion. Organic compounds containing the group -CN are termed either nitriles or cyanides, depending upon the nomenclature used. In the context of this document, total cyanide refers to the summation of the CN group in the different cyanide compounds that exist in aqueous solution. Total cyanide has been referred to operationally as those compounds which are determined by a particular, selected analytical technique. Depending upon the circumstances, these two definitions of total cyanide may not be comparable.

1.1 FREE CYANIDE (see Section 2.3)

Free cyanide, composed of the equilibrium summation of molecular HCN and the cyanide ion, is considered the primary toxic agent in the aquatic environment. In natural waters with a pH of 8.5 or lower, most of the free cyanide is in the form of molecular HCN.

1.2 SIMPLE CYANIDE (see Section 2.4)

Simple cyanides, represented by the formula $M(CN)_x$, exhibit a diverse range of aqueous solubilities and degrees of ionization. Soluble simple cyanides, notably some alkali cyanides, readily release cyanide ion in solution; the cyanide ion may then enter into equilibrium with molecular HCN.

1.3 COMPLEX CYANIDE (see Section 2.5)

Transition metals of the "d" block and their near neighbors combine with cyanide ions to produce various complex cyanides, depending upon such factors as solution strength and stoichiometric proportions. Several metallocyanide compounds dissociate in aqueous solution, thereby generating complex cyanide ions. These ions may, depending upon conditions, further dissociate yielding cyanide ions. In general, complex ions are more stable than the parent metallocyanide and their subsequent dissociation may be relatively small. However, the extent to which a complex cyanide dissociates is of little importance in environmental systems; instead, the important factor is the rate at which cyanide bound in the complex exchanges with the cyanide ion (i.e. free cyanide) in solution.

Toxicity of complex cyanides is usually related to the capacity of complex cyanide ions to release cyanide ions in solution, which then enter into an equilibrium with molecular HCN. Relatively small fluctuations in pH may have a significant effect upon toxicity, as has been demonstrated for the tetracyanonickelate II complex.

1.4 ORGANOCYANIDES (NITRILES) (see Section 2.6)

Organic compounds containing the -CN group can be termed cyanides or nitriles, depending upon the nomenclature used. From the point of view of free cyanide toxicity, many organocyanide compounds are considered relatively innocuous in aqueous systems because of their low chemical reactivity and biodegradability. Most degradative mechanisms involve a cleavage of the CN bond and hence the cyanide ion is not generated. It should be kept in mind, however, that some nitriles are toxic per se, although such toxicity is not attributable to release of the cyanide ion.

Cyanohydrins ($R_2C(OH)CN$) and cyanogenic glycosides ($R_1R_2C(OR_3)CN$), are special classes of nitriles in that under appropriate conditions, they will release cyanide ions (i.e. free cyanide) in solution.

1.5 OTHER RELATED COMPOUNDS (see Section 2.7)

Cyanides (mainly free and simple) may be converted to cyanates (compounds containing the group -OCN) during waste treatment. Aqueous solutions of cyanate hydrolyze to ammonia and bicarbonate. In the presence of reduced sulfur compounds, particularly polysulfide sulfur, cyanides are converted to thiocyanates (compounds containing the group -SCN). Thiocyanates are relatively more stable in aqueous solution than cyanates and may form complexes with a number of elements.

1.6 ANALYTICAL METHODOLOGY (see Section 2.8)

Free cyanide can be selectively determined by variations of gas stripping and aqueous diffusion techniques. Analysis of total cyanides can be accomplished by acid distillation with detection by selective ion electrode, titration or colorimetry. Pretreatment and modifications to the acid distillation step are commonly employed to ascertain some fractions of total cyanide. Such modifications include cyanide amenable to chlorination, weak acid dissociable cyanide and simple versus complex cyanide. Qualitative differences existing between the measured fractions may have some bearing upon their toxicity. A comparison of different analytical methods used to measure various cyanide species is presented in Table 2-4.

2.0 ENVIRONMENTAL DYNAMICS (see Chapter 3.0)

Very little reliable information is available on sources, levels, fates and environmental dynamics of cyanide compounds in dilute aqueous systems.

2.1 SOURCES (see Section 3.2)

The presence of cyanides in natural waters is attributed to both natural processes and man-made activities. Although molecular HCN may be produced naturally by microorganisms as well as from the cyanogenic degradation of glycosides, the environmental significance related to such sources has not been assessed. Compounds containing the cyanide group also enter the aquatic environment via point sources associated with certain industrial activities as well as nonpoint sources associated with the application of materials containing cyanide compounds. Detectable levels of cyanide in the environment (usually measured as some form of the total cyanide) are considered to result from improper management practices such as improper storage, handling and disposal of wastes. The pyrolysis of some cyanide-containing materials leads to the production of hydrogen cyanide; the atmospheric transport, transformation and possible entry into the aquatic environment have not been examined.

2.2 LEVELS (see Section 3.3)

The limited data that are available on the distribution of cyanide compounds in the aquatic environment have usually been obtained using techniques to measure only total cyanide, irrespective of the chemical species that may actually be present in such samples. Fragmentary information indicates that total cyanide concentrations in rural areas are related to the size of the watershed and seasonal runoff. Levels of total cyanide are found to be significantly lower in rural and wilderness waters than in waters of industrial origin.

2.3 FATE IN THE AQUATIC ENVIRONMENT (see Section 3.4)

Very little information has appeared in the literature pertaining to the fate of cyanide compounds in natural waters. Depending upon the cyanide species in question and physicochemical conditions, such interactions as oxidation-reduction, volatilization, hydrolysis, photolysis, dissociation, photoaquation, biodegradation-biogenesis and precipitation-dissolution may be important. With few exceptions, studies have been conducted only under conditions which simulate highly concentrated process water or effluents. Dynamic interactions in an environmental context are virtually unexplored.

Hydrogen cyanide is volatile, having a vapor pressure of 53.1 kPa at 10.2°C and will be in equilibrium with atmospheric HCN approximately according to Henry's Law. Volatilization is expected to be a significant means of free cyanide (and in some cases simple cyanide) removal from concentrated effluents: however, it may be a rate-determining step in dilute aqueous systems. Data on chemical

oxidation and adsorption to solids of molecular HCN in dilute aqueous systems are unavailable. Numerous microorganisms have been identified that can degrade molecular HCN. Microbial degradation has been employed as a biological treatment method; however, acclimation to low levels of cyanides are required in order for the mixed cultures to be effective. The relationship between biological synthesis and degradation of molecular HCN, and its environmental significance, has not been investigated.

If conditions in dilute aqueous systems are such that complex cyanide ions release cyanide ions, which will then enter into equilibrium with molecular HCN, the removal mechanisms indicated above will apply. Numerous metallocyanide complex ions are relatively stable in aqueous solution in the absence of ultraviolet and visible light. However, under certain conditions, photodecomposition, with subsequent release of cyanide ions, will occur. The significance of biological degradation, adsorption to solids and other relevant processes in dilute aqueous solution has not been assessed. It is postulated that some of the various mechanisms involved in the removal of cyanide compounds from the water column may result in localized pools or sinks of such material that, upon changing conditions, may release free cyanide or other cyanide-containing material back into the water column.

3.0 TOXICOLOGY (see Chapter 4.0)

3.1 FREE CYANIDE (see Section 4.2)

The acute mode of action of HCN is limited to binding those porphyrins that contain iron III, such as cytochrome oxidase, hydroperoxidases, and methemoglobin. At lethal levels, cyanide is therefore primarily a respiratory poison and one of the most rapidly effective toxicants known.

Concentrations in the range of 30-150 $\mu g.L^{-1}$ HCN may be acutely toxic and include the lethal thresholds and median tolerance concentrations for most cold- and warm-water fishes. The acute toxicity of HCN can be influenced by several modifying factors; however, temperature appears to be most significant. The influence of temperature is related to the HCN concentrations present. At high, rapidly lethal concentrations, cyanide is more toxic at elevated temperatures whereas at relatively low, slowly lethal levels the opposite is true.

At the chronic level of exposure, metabolic processes other than biological oxidation may be responsible for detrimental effects observed at HCN concentrations as low as 5 $\mu g.L^{-1}$. Various responses of ecological significance have been measured. Reproductive impairment has been demonstrated under continuous chronic exposure to HCN at concentrations as low as 4 $\mu g.L^{-1}$. All such tests have revealed significant detrimental effects upon reproductive processes at approximately 10 $\mu g.L^{-1}$.

Fertilized eggs are relatively resistant to free cyanide prior to blastulation, however, teratogenesis and mortality following blastulation, in addition to mortality of newly hatched larvae, have occurred at concentrations of approximately 60-100 $\mu g.L^{-1}$.

The growth of juvenile fish may be severely affected during chronic exposures to 10 $\mu g.L^{-1}$, although transient growth enhancement has been demonstrated.

Tolerance (e.g. levels affecting growth) may change with body size. Chronic exposure to 10 $\mu g.L^{-1}$ HCN has been shown to elevate resting metabolic rate above basal levels. However, this oxidative stimulation in relatively inactive fish remains unexplained. The active swimming ability required for migration, feeding, and escape from predators is severely affected by free cyanide. An experimental situation simulating anadromous migration revealed osmoregulatory disturbances at 10 $\mu g.L^{-1}$ HCN in fresh water, following a period of several days in salt water.

3.2 OTHER RELATED COMPOUNDS (see Section 4.3)

Few detailed toxicity tests have been conducted into the effects of simple cyanide-containing compounds upon aquatic organisms. Cyanates are believed to be relatively nontoxic although the 96-h LC_{50} for mercuric thiocyanate was determined to be 120 $\mu g.L^{-1}$ for a marine invertebrate and 180 $\mu g.L^{-1}$ for a freshwater fish. Cyanogen chloride is toxic to fish at 40 $\mu g.L^{-1}$.

3.3 METALLO- AND ORGANOCYANIDES (see Section 4.4)

The toxicity of metallocyanides may in part be related to molecular structures, as discussed in Section 2.5 of the text. An inherent toxicity is associated with tetracyanonickelate II, dicyanoargentate I, and dicyanocuprate I. The nickel complex, which may have an accumulative mode of action, has been estimated as lethally toxic to fish at 1.0 $mg.L^{-1}$ as total cyanide. The toxic silver and copper complexes may not be significant in the environment because of their removal by wastewater treatment and their rapid dissociation upon dilution of effluents.

Iron cyanide complexes are extremely stable in the absence of ultraviolet or visible light and are essentially nontoxic. However, they can undergo photolysis under certain circumstances and release toxic levels of free cyanide.

The inherent toxicity associated with cobalt and mercury complexes (other than mercuric thiocyanate) has not been investigated. Toxicity of cyano complexes of chromium, cadmium, zinc, and lead has not

been demonstrated; however, solutions of these complexes may produce toxic levels of free cyanide.

Within the organocyanides is a group of cyanohydrins (e.g. lactonitrile) which may produce sufficient HCN via hydrolysis to elicit toxic responses in short-term exposure situations. Other nitriles are less toxic in acute conditions. For example, the 100-d LC_{50} for rainbow trout was estimated as 1.6 $mg.L^{-1}$ as acrylonitrile. Chronic toxicity has not been adequately investigated.

3.4 MULTIPLE TOXICITY (see Section 4.5)

Binary mixtures of free cyanide plus hexavalent chromium, zinc, or ammonia resulted in more fish lethalities than predicted by either response addition or concentration addition models. However, at sublethal levels, only the NH_3-HCN binary mixture had an interactive effect whereas Cr-HCN and Zn-HCN combinations demonstrated independent modes of action. Binary mixtures of arsenic plus free cyanide may elicit effects at sublethal levels which are predictable according to a response addition model.

RECOMMENDATIONS AND RESEARCH NEEDS

1. There is a continuing debate concerning the applicability of various analytical methodologies for measuring cyanide-containing compounds in the aquatic environment and their ecotoxicological significance. From a toxicological point of view, the measurement of free cyanide (specifically molecular HCN) in natural waters is the best chemical indicator of the immediate toxic effects to fish. Unrefined measurements estimating only "total cyanide" are not toxicologically relevant because the exact chemical nature of the toxicant is not known and hence, in order to estimate the toxicant concentration, the chemical speciation must be computed from total cyanide measurements. From an ecological perspective, one must consider not only free cyanide but also those compounds that under given environmental circumstances may generate free cyanide. In addition, such compounds may have toxicological or ecological impacts *per se*. It is therefore recommended that:

> FREE CYANIDE ($HCN + CN^-$) BE CONSIDERED THE PRIMARY CHEMICAL INDICATOR OF IMMEDIATE TOXIC EFFECTS OF CYANIDE-CONTAINING COMPOUNDS. IT IS FURTHER RECOMMENDED THAT A COMPREHENSIVE AND INTERDISCIPLINARY ASSESSMENT OF CURRENT AND PROMISING ANALYTICAL METHODOLOGIES BE CARRIED OUT. A CONCERTED ATTEMPT SHOULD BE MADE TO DEVELOP ANALYTICAL METHODOLOGIES THAT CAN RELIABLY DIFFERENTIATE BETWEEN THE DIFFERENT FORMS OF CYANIDE AND WHICH CAN BE COMPARED ON AN INTERLABORATORY BASIS.
>
> CYANIDE-CONTAINING COMPOUNDS IN NATURAL WATERS SHOULD BE MONITORED USING REFINED ANALYTICAL TECHNIQUES THAT PROVIDE AN IMPROVED RESOLUTION OF THE FORMS OF CYANIDE IN WATER. WHERE MONITORING OF NATURAL WATERS INDICATES ELEVATED LEVELS OF CYANIDES, SITE-SPECIFIC SAMPLING AND ANALYSIS USING TECHNIQUES THAT IDENTIFY ALL FORMS OF CYANIDE SHOULD BE EMPLOYED.

2. Reliable scientific information regarding the fate of cyanide-containing compounds in natural waters is practically nonexistent. Most currently held opinions regarding this are based upon investigations into the treatment of complex industrial wastes containing cyanides at levels in the $mg.L^{-1}$ range.

Based upon the information obtained from effluent treatment and from a few relatively simple laboratory experiments using very high cyanide concentrations, it has been generally assumed that cyanide has a short residence time in the aquatic environment. However, it is often difficult and misleading to extrapolate such information to the natural environment because of modifying factors such as temperature, pH, sunlight, turbidity and biological activity. The primary conclusion from the relatively few studies involving *in situ* measurements in certain segments of the aquatic environment is that we cannot assume

that various cyanide-containing compounds have a short residence time in natural waters.

Because the current information is clearly inadequate to describe the fate of cyanide-containing compounds in natural waters, it is recommended that:

A. SITE-SPECIFIC SURVEYS BE CONDUCTED IN CANADA, IN CONJUNCTION WITH ROUTINE MONITORING AS RECOMMENDED PREVIOUSLY, TO DELINEATE THE FATE OF CYANIDE-CONTAINING COMPOUNDS IN NATURAL WATERS. THE RELATIVE CONTRIBUTIONS OF BOTH NATURAL AND MAN-MADE SOURCES NEED TO BE EXAMINED. IN ADDITION, CRITICAL EXPOSURE ROUTES FOR AQUATIC ORGANISMS NEED TO BE IDENTIFIED.

B. STANDARDIZED MODEL ECOSYSTEMS WITH MEASURABLE PHYSICOCHEMICAL PARAMETERS THAT SIMULATE VARIOUS SEGMENTS OF THE AQUATIC ENVIRONMENT NEED TO BE DESIGNED SO THAT RELEVANT STUDIES ON THE AQUATIC FATE OF CYANIDE COMPOUNDS MAY BE CONDUCTED.

3. The toxicity of free cyanide at the lethal and sublethal levels of exposure has been extensively studied in the laboratory, using freshwater fish that had not been previously exposed to cyanide-containing compounds. In addition, fixed concentrations of toxicant were usually used for the duration of the tests. Although such test conditions are not found in the natural environment, the investigations have indicated the toxicologically effective range of free cyanide concentrations and permitted a qualitative description of specific effects in fish. In order to fill gaps in scientific knowledge, it is recommended that:

A. THE TOXICITY OF CYANIDE-CONTAINING COMPOUNDS TO FRESHWATER ORGANISMS OTHER THAN FISH BE EXAMINED AT LEAST AT THE LETHAL LEVEL OF EXPOSURE.

B. IN VIEW OF THE POTENTIAL FOR RELEASE OF CYANIDE-CONTAINING COMPOUNDS TO THE MARINE ENVIRONMENT, TOXICOLOGICAL STUDIES USING MARINE ORGANISMS SHOULD BE CARRIED OUT.

C. EXPERIMENTAL CONDITIONS USED IN TOXICOLOGICAL STUDIES SHOULD MORE CLOSELY SIMULATE CANADIAN ENVIRONMENTAL CONDITIONS. USING RELIABLE SCIENTIFIC DATA GENERATED FROM STUDIES OF ENVIRONMENTAL DYNAMICS, REALISTIC PROTOCOLS FOR THE DEVELOPMENT OF TOXICOLOGICAL EXPERIMENTS SHOULD BE CONSIDERED.

D. STUDIES INVESTIGATING THE EFFECTS OF CYANIDE-CONTAINING COMPOUNDS ON AQUATIC COMMUNITIES, AS OPPOSED TO ISOLATED ORGANISMS, SHOULD BE CARRIED OUT.

E. INTERMEDIARY METABOLISM IN ORGANISMS EXPOSED TO CYANIDE-CONTAINING COMPOUNDS SHOULD BE INVESTIGATED.

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1.0 INTRODUCTION

The widespread occurrence of numerous cyanide-containing compounds in terrestrial and aquatic ecosystems has been known for many years, and has been attributed to both natural and man-made sources. Because several cyanide-containing compounds are known to be very toxic to resident biota under certain environmental conditions, there is concern about the potential detrimental effects from the release of elevated levels of such compounds to the aquatic environment. The Subcommittee on Water of the NRCC Associate Committee on Scientific Criteria for Environmental Quality requested that scientific information be gathered and reviewed on the fate and effects of cyanide-containing compounds in the Canadian aquatic environment.

The following document briefly reviews the physicochemical properties of cyanide compounds along with analytical methodologies used for water samples. The fate of cyanide-containing compounds in the aquatic environment is also examined. The majority of information contained in this document refers to the toxicity of molecular HCN to freshwater fish, especially at the chronic level of exposure. Where available, information on the effects of cyanides on other aquatic organisms is included. This document does not treat the topic of cyanide in drinking water for ingestion by man or nonaquatic animals, nor the effects of cyanides on terrestrial organisms.

2.0 CHEMISTRY

2.1 TERMINOLOGY

Cyanides comprise a diverse group of inorganic and organic compounds characterized by the chemical group -C≡N. Cyanides may be classified according to their physicochemical properties as free, simple, complex and organic cyanides (Kelada et al. 1978). Cyanide ion refers to the single, free anion (CN^-) per se, excluding all other cyanide species (Doudoroff 1976). Molecular HCN (hydrocyanic acid, hydrogen cyanide, HCN) refers to cyanide in the form of an uncharged, intact molecule. Free cyanide refers to the summation of molecular HCN and the cyanide ion in aqueous solution, irrespective of their sources in water. Simple cyanides are those compounds that dissociate directly in water to a cation and the cyanide ion, with no soluble intermediates. Complex cyanide is that cyanide which is incorporated into a complex or complexes. Depending upon conditions, complexes may dissociate in water to a cation and an anion composed of two or more different molecules, one of which is cyanide. The anion is termed a complex ion and may be subject to further dissociation, thereby releasing cyanide ions (E.A. 1979). Organic compounds containing the group -C≡N are termed nitriles or cyanides, depending upon the nomenclature used (IUPAC 1979).

The term total cyanide has been used to convey different ideas in the literature (Doudoroff 1976). From an operational viewpoint, total cyanide comprises all cyanide species or groups determinable by a particular, selected analytical method. Total cyanide also refers to the summation of the CN group in all the different forms of cyanide that exist in aqueous solution. Terms such as total cyanide, oxidizable cyanide, cyanide amenable to chlorination, cyanide amenable to ozonation etc. reflect the corresponding methods of analysis (Kelada et al. 1978).

It must be borne in mind that cyanide may exist in several different forms in the aquatic environment, depending upon a variety of physical and chemical modifying factors. The form of "cyanide" in water has a direct influence on such considerations as chemodynamics, analytical methodologies and ecotoxicology.

Several reviews of the literature pertaining to the chemistry of cyanide have been published. These include the works of Griffith (1962), Ford-Smith (1964), Sharpe (1976), Towill et al. (1978), International Environmental Consultants (I.E.C. 1979), and Ecological Analysts (E.A. 1979). Organocyanide chemistry is the subject of texts by Migrdichian (1947) and Rappoport (1970), and is included in the review by Towill et al. (1978).

It is not the intention of this document to duplicate these excellent reviews. Instead, a brief summary of cyanide chemistry in aqueous media is presented along with an overview of the chemistry of some of the cyanide compounds that may be present in the aquatic environment.

2.2 BONDING PROPERTIES

The cyanide anion (CN^-), consisting of a carbon and nitrogen atom triply bonded, has the electronic configuration $(\sigma ls)^2 (\sigma^* ls)^2 (\sigma 2s)^2 (\sigma^* 2s)^2 (\sigma 2p_x)^2 (\pi 2p_y)^2 (\pi 2p_z)^2$ (Sharpe 1976). Outwardly the cyanide group appears as a cylinder of π-electrons capped on one end by an unshared pair of electrons (Sheppard 1970). The relatively large bond moment of the -CN group, due mainly to the lone pair orbital of electrons centered on the nitrogen atom and directed along the CN-axis, is responsible for much of the reactivity of the inorganic and organic cyanide compounds.

The coordination ability of both metallo- and organocyanides is fundamentally related to the electronic structure of the cyanide group. In relatively simple systems, bonding consists of a σ-bond and two π-bonds, giving the linear configuration X-CN, where X is a certain metal or organic moiety. In addition, it is generally accepted that the cyanide group acts as a σ-electron donor and a π-electron acceptor (Sharpe 1976).

2.3 FREE CYANIDE

Hydrocyanic acid (HCN, molecular cyanide) is a small, linear, and uncharged molecule which can readily penetrate biological membranes. HCN is infinitely soluble in aqueous media in equilibrium with negligible or low concentrations of the cyanide ion (CN^-):

$$\frac{[CN^-][H^+]}{[HCN]} = K_{eq.} = 10^{-9.4} \text{ at } 25°C \qquad (2\text{-}1)$$

In general, environmental factors have a minor influence upon this equilibrium. For example, at 10°C and pH 6.0, more than 99% of the cyanide present exists as HCN. The proportion of HCN is expected to decrease slightly to 92% at pH 8.5. Under extreme conditions of very high pH a significant portion of free cyanide would exist as CN^-. The equilibrium constant varies slightly with temperature: from $10^{-9.6}$ at 10°C to $10^{-8.9}$ at 40°C (Sharpe 1976). Extrapolating to a temperature more characteristic of northern Canada or winter conditions in southern Canada, approximately 94% of free cyanide would be in the form of HCN at 4°C and pH 8.5.

2.4 SIMPLE CYANIDE

Simple cyanides may be represented by the formula $M(CN)_x$, where M is an alkali, such as calcium, sodium, potassium or a metal, and x, the valence of M, represents the number of cyano groups present in the molecule (APHA-AWWA-WPCF 1981). Simple cyanides exhibit a considerable range of aqueous solubilities and degrees of ionization which govern the amount of cyanide ion released in aqueous solution (see Table 2-1). Soluble compounds, especially the alkali cyanides, dissociate in water to give the cyanide ion.

Table 2-1. Physicochemical properties of some cyanide compounds and related compounds.[a]

Compound	Formula	Molecular Weight	Density or Specific Gravity	Melting Point (°C)	Boiling Point (°C)	Solubility in water ($mg.L^{-1}$)
cadmium cyanide	$Cd(CN)_2$	164.45		d 200		1.7×10^4
cadmium ferrocyanide	$Cd_2Fe(CN)_6.H_2O$					i
cobalt II cyanide dihydrate	$Co(CN)_2.2H_2O$	147.00	1.872 (anhydr.)	$-2H_2O$,280	d 300	41.8
cobalt II ferricyanide	$Co_3[Fe(CN)_6]_2$	600.71				i
cobalt II ferrocyanide	$CO_2Fe(CN)_6.xH_2O$					i
cobalt thiocyanate	$Co(SCN)_2.3H_2O$	229.14		$-3H_2O$,105		s
copper I cyanide	CuCN	89.56	2.92	473 (in N_2) d		i
copper II cyanide	$Cu(CN)_2$	115.58		d		i
copper I ferricyanide	$Cu_3Fe(CN)_6$	402.57				i
copper II ferricyanide	$Cu_3[Fe(CN)_6]_2.14H_2O$	866.74				i
copper II ferrocyanide	$Cu_2Fe(CN)_6.xH_2O$					i
copper II thiocyanate	$Cu(SCN)_2$	179.7		d 100		d
cyanogen	$(CN)_2$	52.04	2.335 $g.L^{-1}$ (gas)	-27.9	-20.7	4500 $cm^3.L^{-1}$
cyanogen chloride	CNCl	61.47	1.186 $g.L^{-1}$ (gas)	-6	12.66	sl s
hydrogen cyanide	HCN	27.03	0.901 $g.L^{-1}$ (gas)	-14	26	∞
iron II thiocyanate	$Fe(SCN)_2.3H_2O$	226.06		d		vs
iron III thiocyanate	$Fe(SCN)_3$	230.09				vs
lead cyanide	$Pb(CN)_2$	259.23				sl s
lead ferricyanide	$Pb_3[Fe(CN)_6]_2.5H_2O$	1135.55		$-H_2O$, 110-120d		sl s
lead ferrocyanide	$Pb_2Fe(CN)_6.3H_2O$	680.38		$-H_2O$,100		i
mercury II cyanide	$Hg(CN)_2$	252.63	3.996	d		9.3×10^4
lead thiocyanate	$Pb(SCN)_2$	323.35	3.82	d 190		5×10^2
nickel cyanide	$Ni(CN)_2$	110.75				i
nickel cyanide tetrahydrate	$Ni(CN)_2.4H_2O$	182.81		$-4H_2O$,200	d	i
nickel ferrocyanide	$Ni_2Fe(CN)_6.xH_2O$		1.892			i
potassium cyanide	KCN	65.12	1.52	634.5		5×10^5
potassium cyanoargentate I	$K[Ag(CN)_2]$	199.01	2.36			2.5×10^5

Table 2-1. (cont'd)

Compound	Formula	Molecular Weight	Density or Specific Gravity	Melting Point (°C)	Boiling Point (°C)	Solubility in water ($mg.L^{-1}$)
potassium ferricyanide	$K_3Fe(CN)_6$	329.26	1.85	d		3.3×10^5
potassium ferrocyanide	$K_4Fe(CN)_6.3H_2O$	422.41	1.85	$-3H_2O$,70	d	2.78×10^5
potassium thiocyanate	KNCS	97.18	1.886	173.2	d 500	1.77×10^6
silver cyanide	AgCN	133.84	3.95	d 320		0.23
silver ferricyanide	$Ag_3Fe(CN)_6$	535.56				0.66
silver ferrocyanide	$Ag_4Fe(CN)_6.H_2O$	661.45				i
silver thiocyanate	AgSCN	165.95		d		0.21
sodium cyanide	NaCN	49.01		563.7	1496	48×10^5
sodium cyanoaurite	$NaAu(CN)_2$	272.02				s
potassium cyanoaurate	$K[Au(CN)_2]$	288.1	3.45			1.43×10^5
potassium cyanoaurate III	$K[Au(CN)_4.1\frac{1}{2}H_2O]$	367.16		d 200		s
potassium cyanocadmate II	$K_2[Cd(CN)_4]$	294.68	1.85			3.3×10^5
potassium cyanochromate III	$K_3[Cr(CN)_6]$	325.41	1.71			3.09×10^5
potassium cyanocobaltate II	$K_4[Co(CN)_6]$	371.42				s
potassium cyanocobaltate III	$K_3[Co(CN)_6]$	332.32	1.906	d		s
potassium cyanocuprate I	$K_3[Cu(CN)_4]$	284.92		d		vs
potassium cyanonickelate II	$K_2[Ni(CN)_4.H_2O]$	259.00	1.875	$-H_2O$,100		s
sodium cyano-cuprate I	$NaCu(CN)_2$	138.57	1.013	d 100		s
sodium ferricyanide	$Na_3Fe(CN)_6.H_2O$	298.92				1.8×10^5
sodium ferro-cyanide	$Na_4Fe(CN)_6.10H_2O$	484.04	1.458			3.19×10^5
sodium thiocyanate	NaSCN	81.07		287		1.4×10^6
zinc cyanide	$Zn(CN)_2$	117.41	1.852	d 800		5
zinc ferrocyanide	$Zn_2Fe(CN)_6$	342.69	1.85			i

[a] Taken from CRC Handbook of Chemistry and Physics 1979/80.

d decomposes

i insoluble

sl s slightly soluble

s soluble

vs very soluble

∞ infinitely soluble

For example:

$$NaCN \rightarrow Na^+ + CN^- \qquad (2\text{-}2)$$

$$CN^- + H_2O \rightleftarrows HCN + OH^- \qquad (2\text{-}3)$$

2.5 COMPLEX CYANIDE

Inorganic cyanide complexes are formed almost exclusively with the transition metals of the "d" block and their near neighbors, such as Zn, Cd and Hg, and have the general formula $[M^{n+}(CN)_x]^{(x-n)\pm}$. Mixed complexes, particularly of the type $[M^{n+}(CN)_5X]^{n-5}$, where X may be H_2O, NH_3, CO, NO, H or a halogen, are also well known. It has been observed that there are 28 elements that form complex cyanide compounds and that there are over 68 oxidation states of these elements capable of forming complex cyanides under certain conditions (Broderius 1973). Hence, there exists the possibility of a multitude of different metallocyanides that may occur in aquatic ecosystems, all of which possess different chemical and physical properties. Little information is available on the predominant chemical species present in the Canadian aquatic environment; the following discussion is a brief examination of some of the cyanide compounds for which aquatic chemistry data exist. In addition, certain cyanide compounds have been selected as models for illustrating structure-biological activity relationships.

Under certain conditions, complex metallocyanides will dissociate in water, thereby generating a complex cyanide ion which may further dissociate releasing cyanide ions. In general, many complex ions are more stable than their original compounds and hence their subsequent dissociations may be relatively small. Although a simple equation may be written to describe the overall dissociation of a complex cyanide ion, such simple, one-step reactions are rare in natural water systems (Bockris 1977). The actual process usually involves a number of intermediate steps and to appreciate the quantitative effect of altering conditions on an equilibrium, all intermediate species need be considered from a kinetic point of view. Although one species could predominate at any given time, other species could also be present. Unfortunately, very little work has been done on the elucidation of all intermediate reactions of the metallocyanide studies thus far and, hence, one must rely upon overall or cumulative dissociation or equilibrium constants for many complex ions.

According to Broderius (1973) the extent to which a complex cyanide dissociates is of little importance in environmental systems; the important factor is the rate at which cyanide bound in the complex exchanges with free cyanide in solution:

$$[M(CN)_x]^{-a} + H_2O \rightleftarrows [M(CN)_{x-1}\cdot H_2O]^{-a+1} + CN^- \qquad (2\text{-}4)$$

The rate of exchange is independent of total cyanide concentration and is governed by the pH of the medium and the light intensity (MacDiarmid and Hall 1953; 1954).

2.5.1 NICKEL

Potassium cyanonickelate II, $K_2[Ni(CN)_4.H_2O]$ and the equivalent sodium complex are soluble in water (Table 2-1) and disproportionate to give the $[Ni(CN)_4]^{-2}$ ion.

Cyanonickelate II exists in equilibrium with cyanide and nickel ions:

$$[Ni(CN)_4]^{-2} \rightleftarrows Ni^{2+} + 4CN^- \qquad (2\text{-}5)$$

$$4CN^- + 4H^+ \rightleftarrows 4HCN \qquad (2\text{-}6)$$

The value of the equilibrium constant changes with temperature (Sharpe 1976) and may be subject to variation from as yet unknown sources (Broderius 1973); however, only a representative value is included in Table 2-2 for chemical speciation calculations. Formation and dissociation of the complex are very slow; as much as ten days may be required to achieve dissociation equilibrium (Broderius 1973). Hence, equation 2-5 is actually the summation of several reactions, each with its own dissociation constant.

Dissociation of the tetracyanonickelate complex produces cyanide ions which will, in turn, form HCN. The greater tendency toward HCN formation at lower pH will eventually drive a dilute Ni-CN system to complete dissociation (Doudoroff 1976). Under such conditions, a solution of high toxicity may result. In alkaline environments, most of the free cyanide will be complexed with nickel, making the solution less toxic. Whereas pH has a minor influence upon the toxicity of free cyanide, minor changes in pH markedly affect the toxicity of some metallocyanides. For example, Doudoroff (1976) noted that the maximum effect of pH on the tetracyanonickelate complex in the range 6.5-8.0 occurred from 7.8 to 7.4, corresponding to a 10- to 13-fold increase in toxicity, expressed as total cyanide concentration.

A relatively low toxicity has been ascribed to the tetracyanonickelate II complex <u>per se</u> (Broderius 1973):

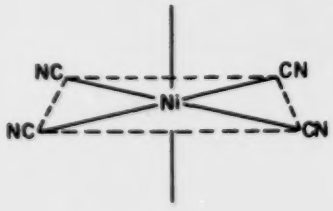


Table 2-2. Data pertinent to metallocyanide speciation.

Compound	Formula	Shape	Log cumulative equilibrium constant[a]	Reference
dicyanoargentate I	$[Ag(CN)_2]^{-1}$	linear	-18.7	Broderius (1973)
tricyanoargentate I	$[Ag(CN)_3]^{-2}$		-20.25	Sharpe (1976)
dicyanocuprate I	$[Cu(CN)_2]^{-1}$	equilateral triangular	-23.9	Sharpe (1976)
tricyanocuprate I	$[Cu(CN)_3]^{-2}$	distorted tetrahedral planar	-29.2	Sharpe (1976)
tetracyanocuprate I	$[Cu(CN)_4]^{-3}$	tetrahedral	-30.7	Sharpe (1976)
tetracyanonickelate II	$[Ni(CN)_4]^{-2}$	square planar	-31	Broderius (1973)
hexacyanoferrate II	$[Fe(CN)_6]^{-4}$	octahedral	-47	Broderius (1973)
hexacyanoferrate III	$[Fe(CN)_6]^{-3}$	octahedral	-52	Broderius (1973)
cyanomercurate II	$Hg\ CN^{+}$	linear	-17	Sharpe (1976)
dicyanomercurate II	$Hg(CN)_2$		-32.75	Sharpe (1976)
tricyanomercurate II	$[Hg(CN)_3]^{-1}$		-36.31	Sharpe (1976)
tetracyanomercurate II	$[Hg(CN)_4]^{-2}$	tetrahedral	-38.97	Sharpe (1976)
tetracyanocadmate (II)	$[Cd(CN)_4]^{-2}$		16.8	Smith (1976)
dicyanaurate (I)	$[Au(CN)_2]^{-1}$		38.3	Dean (1973)
tetracyanozincate (II)	$[Zn(CN)_4]^{-2}$		16.7	Smith (1976)

[a] Cumulative equilibrium constant - the equilibrium constant for the complete dissociation of the complex ion, irrespective of the intermediate steps.

In biological systems, the complex is relatively stable and would not be expected to release CN^- ions internally (assuming the integrity of the blood buffering system is unaffected). However, the complex's mode of action may be a function of its ability to coordinate with two additional ligands and form octahedral complexes (Cotton and Wilkinson 1972) at the critical site. This concurs with observed symptoms of tetracyanonickelate II toxicity (Doudoroff 1956) which are in some ways similar to Ni^{2+} toxicity (see Section 4.1), but are uncharacteristic of cyanide toxicity.

2.5.2 SILVER

Silver cyanides dissolve readily in water, with the exception of the iron II complex ($Ag_4Fe(CN)_6 \cdot H_2O$) (Table 2-1). Aqueous species include $[Ag(CN)_2]^{-1}$, $[Ag(CN)_3]^{-2}$ and $[Ag(CN)_4]^{-3}$ which have repeatedly been shown to obey the following equilibria at zero ionic strength (Sharpe 1976):

$$[Ag(CN)_3]^{-2} \rightleftarrows [Ag(CN)_2]^{-1} + CN^- \qquad K_{eq} = 10^{-1.55} \qquad (2\text{-}7)$$

$$[Ag(CN)_2]^{-1} \rightleftarrows Ag^+ + 2CN^- \qquad K_{eq} = 10^{-20.44} \qquad (2\text{-}8)$$

However, the complex ions $[Ag(CN)_3]^{-2}$ and $[Ag(CN)_4]^{-3}$ are only stable in concentrated cyanide solutions. The dicyanoargentate I complex will rapidly exchange with labelled HCN in solution (Sharpe 1976). The true equilibrium value to be used in chemical speciation calculations should be 1.94×10^{-19} (Table 2-2) (Broderius 1973; Doudoroff 1976).

The complex $[Ag(CN)_2]^{-1}$ is very toxic to fish (Doudoroff *et al.* 1966; Broderius 1973):

$$N{\equiv}C - Ag^- - C{\equiv}N$$

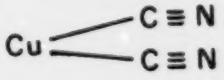
Relatively high toxicity may be attributed to its small molecular dimensions and linear shape, which would facilitate membrane penetration. Symptoms of dicyanoargentate I intoxication are similar to those of the silver ion and may be the consequence of silver ion release from the complex by dissociation, or bonding to the CN-complexed silver atom at the critical site.

However, the dicyanoargentate I ion exists only under specific conditions with respect to a) the mole ratio between silver and cyanide, b) the total concentrations of silver and cyanide, and c) the presence of competing metals. The possible occurrence of the dicyanoargentate I complex is made more remote by efficient recovery from process water (e.g. metallurgic, photofinishing).

2.5.3 COPPER

Only the $+^1_1$ oxidation state of copper occurs in water-soluble coordination complexes with cyanide (Table 2-1). Potassium cyanocuprate I and sodium cyanocuprate I are soluble, whereas copper II thiocyanate is not stable in water. Common aqueous species include $[Cu(CN)_2]^{-1}$, $[Cu(CN)_3]^{-2}$ and $[Cu(CN)_4]^{-3}$ whose equilibrium constants are given in Table 2-2. When a copper cyanide solution was prepared having the mole ratio CN:Cu = 2.0, equilibrium was established within 2-3 days (Broderius 1973). However, equilibrium was not attained at CN:Cu mole ratios of 2.5 or 3.0 after an extended period. This finding was interpreted by Doudoroff (1976) as a sign of the slow formation and dissociation of the $[Cu(CN)_3]^{-2}$ complex in comparison with $[Cu(CN)_2]^{-1}$. When both species exist in a solution, rapid exchange occurs with labelled aqueous cyanide (Sharpe 1976); possibly $[Cu(CN)_2]^{-1}$ is largely responsible for this exchange.

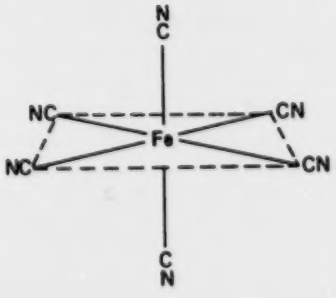
The dicyanocuprate I complex, $[Cu(CN)_2]^{-1}$, is toxic to fish (Doudoroff 1976). The complex elicits symptoms characteristic of copper toxicity which are presumably related to its equilateral triangular shape (Sharpe 1976):



Dicyanocuprate I may be a prominent toxicant under alkaline conditions with total cyanide levels greater than 1 $mg.L^{-1}$. High levels of copper are often found in cyanide-containing effluents.

2.5.4 IRON

Iron complexes of limited solubility (Table 2-1) are hexacyanoferrate II, $[Fe(CN)_6]^{-4}$, usually referred to as ferrocyanide, and hexacyanoferrate III, $[Fe(CN)_6]^{-3}$, commonly known as ferricyanide. These complexes are extremely stable, which is in part attributable to their d-orbital electronic configuration, thereby maximizing both σ and π bonding. The iron complexes therefore exist in equilibrium with negligible amounts of cyanide ion and iron (Table 2-2). High stability together with the fact that the iron atom is completely surrounded by cyanide ligands in an octahedral configuration:



contributes to the complexes' lack of toxicity. However, ultraviolet or visible light will liberate cyanide ions at high quantum yields (Burdick and Lipschuetz 1950; Shirom and Stein 1971a, 1971b; Sharpe 1976; Broderius and Smith 1980). The extent of photolysis is dependent on several factors including the degree and duration of illumination, latitude, time of day and year, and the depth and turbidity of the water. Generally in deep, turbid or shaded waters, photolysis can be assumed to be negligible (Broderius and Smith 1980). However, in clear shallow waters, toxic levels of HCN may be produced from nontoxic iron-cyanide complexes (Burdick and Lipschuetz 1950).

2.5.5 MERCURY

Mercury II cyanide dissolves readily in water (Table 2-1). Aqueous species include mono-, di-, tri-, and tetracyanomercurate II complexes whose equilibrium constants are listed in Table 2-2. The times required to achieve equilibrium have not been reported, but rapid exchange occurs with labelled cyanide (Sharpe 1976). Under sulfidic, reducing conditions in the environment, mercury II sulfide may precipitate with the release of cyanide ions. Toxicity associated with one or more of the mercury II cyanide complexes would not be unexpected, but has not been determined thus far.

2.5.6 CHROMIUM

Although potassium cyanochromate III is very soluble in water (Table 2-1), the resulting hexacyanochromate III ion is thermodynamically unstable and hydrolyzes very slowly according to the overall equation (Sharpe 1976):

$$3H^+ + [Cr(CN)_6]^{-3} + 3H_2O \rightleftarrows Cr(OH)_3 + 6HCN \qquad (2\text{-}9)$$

In light, photoaquation causes essentially the same reaction to occur but at a faster rate. The primary quantum yield is approximately 0.1. Wastewater treatment using strong alkali is expected to aid the chemical decomposition of $[Cr(CN)_6]^{-3}$. Toxicity associated with chromium complexes has not been reported although complete dissociation of the complex has been assumed in toxicity tests (Broderius and Smith 1979).

2.5.7 COBALT

In the +2 oxidation state, $[Co(CN)_5]^{-3}$ and $Co(CN)_2$ are among the species which may occur. The complex $[Co(CN)_5]^{-3}$ decomposes under acidic conditions to Co^{+2} and HCN. In addition, the pentacyanocobalt II complex is subject to oxidation, addition, aquation, and reactions with organohalides producing organocyanides. In the +3 oxidation state, $[Co(CN)_6]^{-3}$ is an extremely stable complex which resists oxidation and is therefore difficult to remove from industrial wastewaters. However,

it will undergo photoaquation producing $[Co(CN)_5H_2O]^{-2}$ + HCN with a quantum yield of 0.2-0.3. The toxicity of cobalt-cyanide complexes has not been determined.

2.5.8 CADMIUM, ZINC, LEAD

Tetracyano- complexes of cadmium II and zinc II, in addition to complexes formed with lead (e.g. $Pb(CN)_2$), may exist in highly concentrated process waters but are expected to dissociate readily during in-plant effluent treatment. Equilibrium constants (Table 2-2) indicate that the complexes will dissociate upon effluent dilution and therefore will be a significant source of free cyanide and metals (Doudoroff 1976).

2.6 ORGANOCYANIDES (NITRILES)

According to IUPAC (1979), organic compounds containing the group -CN are termed nitriles (substitutive nomenclature) or cyanides (radicofunctional nomenclature). A multitude of organic compounds containing the CN group exist and it is not the intention of this document to exhaustively review the chemistry of all such compounds. Because of the general lack of data on the presence of organic cyanides in the aquatic environment, the following discussion will focus upon examples of those compounds that may occur, from either natural or anthropogenic sources in receiving waters (cf. Table 2-3). Based on current physicochemical data, such compounds may be assessed for their potential to release free cyanide in water. It must also be emphasized that the presence in aquatic ecosystems of such compounds may be environmentally significant because they may be inherently toxic or possess some other adverse ecological effect.

2.6.1 LACTONITRILE

Compounds such as lactonitrile are termed cyanohydrins and constitute a special class of nitriles having the general formula $R_2C(OH)CN$. Under certain environmental conditions, cyanohydrins can decompose to form the cyanide ion and molecular HCN. Such cyanohydrins have been found to be about as acutely toxic as simple cyanide salts (e.g. KCN, NaCN) (Doudoroff 1976). Such toxicity has been related to the release of free cyanide. For example, lactonitrile, having a vapor pressure of 0.1 kPa at 58.8°C and 1.3 kPa at 102°C (CRC 1979-1980), is soluble in water where it hydrolyzes completely and relatively rapidly (< 3 h) to form HCN (Henderson et al. 1961).

2.6.2 ACRYLONITRILE

Acrylonitrile is a low-density, volatile liquid with vapor pressures of 6.6 kPa at 8.7°C and 13.3 kPa at 23.6°C (Miller and

Table 2-3. Physicochemical properties of some organocyanides[a].

Compound	Formula	Molecular Weight	Melting Point (°C)	Boiling Point (°C)	Density	Solubility in water
propionitrile	CH_3CH_2CN	55.08	-92.89	97.35	0.7818	v
acetonitrile	CH_3CN	41.05	-45.72	81.6	0.7857	∞
acrylonitrile	CH_2CHCN	53.06	-83.5	77.5-9	0.8060	s
benzonitrile	C_6H_5CN	103.13	-13	190.7	1.0102	sl
malononitrile	$CH_2(CN)_2$	66.06	32	218-219	1.1910	s
lactonitrile	$CH_3CH(OH)CN$	71.08	-40	182-184	0.8877	∞
adiponitrile	$NC(CH_2)_4CN$	108.15	1	295	0.9676	sl
2-hydroxy, 2-methyl-propanonitrile	$(CH_3)_2C(OH)CN$	85.11	-19	82	0.932	v
1-cyanoguanidine	$NH:C(NH_2)\ NHCN$	84.08	211-212	d	1.404	s
malonic acid mononitrile	$NCCH_2CO_2H$	85.06	70-71	108		s
2-naphthonitrile	$C_{11}H_7N$	153.19	66	306.5	1.0939	sl
3-phenanthronitrile	$C_{15}H_9N$	203.27	102	-	-	-

[a] Taken from CRC Handbook of Chemistry and Physics 1979/80.

d decomposes

sl slightly soluble

s soluble

v very soluble

∞ infinitely soluble

Villaume 1978). Although moderately soluble in water (Table 2-3), it is relatively unreactive. Chemical decomposition is expected to be negligible in surface waters, although certain metal catalysts (i.e. Ni II, Co II, Cu II, Rh III) in sludges and sediments are capable of promoting acrylonitrile conversion to amides (Butler et al. 1978; Clark and Hay 1974; Diamond et al. 1974; Zanella and Ford 1975).

Microorganisms degrade acrylonitrile to ammonia and acrylic acid. In addition, wastewaters can be treated chemically by alkaline hydrolysis which produces amides and ammonia. The low temperature (i.e. 250°C) burning of the acrylonitrile monomer does not yield hydrogen cyanide gas (Miller and Villaume 1978) but at increased temperatures of pyrolysis, increasing amounts of HCN are formed, and at 500°C as much as 3 g HCN can be formed per litre acrylonitrile. Thermal combustion of acrylonitrile plastics (i.e. polyacrylonitrile, acrylonitrile-styrene, and acrylonitrile-butadiene-styrene) yields 2-16 g HCN per 100 g plastic material (Miller and Villaume 1978).

2.6.3 ACETONITRILE

Acetonitrile is a low-density, volatile liquid with vapor pressures of 5.3 kPa at 7.7°C and 13.3 kPa at 27°C (CRC 1979-1980). Acetonitrile is infinitely soluble in water (Table 2-3) although chemical decomposition reactions are usually negligible (Henderson et al. 1961; Doudoroff 1976). However, as in the case of acrylonitrile, metal catalysis in sludges and sediments may cause amide formation. Acetonitrile is biodegradable and bacterial end products include acetic acid and ammonia (DiGeronimo and Antoine 1976).

2.6.4 BENZONITRILE

Benzonitrile is a nonvolatile liquid which is slightly soluble in water (Table 2-3). The physicochemical characteristics suggest that benzonitrile would be a persistent substance in aquatic ecosystems. Studies by Hsu and Camper (1973) and DiGeronimo and Antoine (1976) support the hypothesis that benzonitrile is not susceptible to microbial attack. However, there is some controversy concerning the resistance of benzonitrile to degradation. Metal-catalyzed hydrolysis in sludges and sediments and biodegradation by microorganisms are thought to occur (Miller and Villaume 1978; Ludzack et al. 1958).

Many benzonitrile derivatives demonstrate phytotoxic properties and have been utilized as herbicides. In particular, the growth of aquatic weeds has been controlled by 2,6-dichlorobenzonitrile application (Merck 1976). Milborrow and Harthoorn (1975) reported other nontoxic compounds which become effective herbicides when converted to 2,6-dichlorobenzonitrile by microorganisms. Further substitution of benzonitrile yields various chlorinated organophosphate and chlorinated

hydrocarbon biocides such as cyanophenphos, ($C_{15}H_{14}NO_2PS$), cyanophos, ($C_9H_{10}NO_3PS$), and tetrachloroisophthalonitrile, ($C_8Cl_4N_2$).

2.6.5 ADIPONITRILE

Adiponitrile is thought to be a slightly soluble liquid (CRC 1979-1980) although concentrations up to 1300 $mg.L^{-1}$ have been reported in water (Henderson et al. 1961). Adiponitrile apparently hydrolyzes to form amides, but not HCN (Henderson et al. 1961).

2.6.6 MALONONITRILE

Malononitrile is a solid below 32°C and dissolves readily in water. However, the compound is essentially unreactive under the conditions present in most natural waters. Malononitrile hydrazide is manufactured for use as an antitubercular and antihelminthic agent (Merck 1976). A chemical used for crowd control, σ-chlorobenzylidene malononitrile, dissociates completely in water releasing malononitrile.

2.6.7 NATURALLY-OCCURRING ORGANOCYANIDES

Numerous plants are able to synthesize glycosides having one to several CN groups incorporated into the molecule. Such compounds are termed cyanogenic glycosides and have the general formula $R_1R_2C(OR_3)CN$. There are about 28 different cyanoglycosides synthesized and stored in various tissues of many kinds of plants, including over 1000 species, 100 families and 500 genera (Conn 1979). The ecological significance of these various organocyanides as a source of cyanide in surface waters has not been fully appreciated.

2.7 OTHER RELATED COMPOUNDS

Cyanogen (CN_2) is soluble in and reacts with water to produce molecular HCN (Kleinberg et al. 1960). When chlorine (or bromine) is added to a solution containing free cyanide or some simple cyanides, oxidation takes place producing cyanogen chloride (or a cyanogen halide) (White 1972). For example:

$$NaCN + Cl_2 \rightarrow CNCl + NaCl \qquad (2\text{-}10)$$

In the presence of alkali, cyanogen chloride is converted to cyanate. For example:

$$CNCl + 2\ NaOH \rightarrow NaCNO + NaCl + H_2O \qquad (2\text{-}11)$$

Inorganic cyanates are compounds containing the group -OCN; aqueous solutions of cyanates hydrolyze to ammonia and the corresponding

bicarbonate (Towill et al. 1978). Resnick et al. (1958) found that cyanates were relatively stable at pH 7 in aerobic water at 20°C for up to 10 days; however, decomposition accelerated at lower pH values. Thiocyanates, compounds containing the group -SCN, are relatively more stable than cyanates and form complexes with numerous elements, usually coordinating through the nitrogen atom to the first transition series metals and through the sulfur atom to the second and third transition series metals (Cotton and Wilkinson 1972). Alkali and alkaline earth thiocyanates usually readily dissolve in water. Free cyanide, in the presence of reduced sulfur compounds, notably polysulfide sulfur, may be converted to thiocyanate (Luthy and Bruce 1979; Luthy et al. 1979). Thiocyanates appear to be relatively stable in aqueous solution (Kelada et al. 1978).

2.8 ANALYTICAL METHODOLOGY

Because cyanide may exist in many different forms in the aquatic environment, numerous analytical techniques have been developed to measure them, making interlaboratory comparisons difficult. These techniques are based on operational definitions of cyanide i.e. dissociable, weak-acid dissociable, cyanide amenable to chlorination, total, etc. rather than on individual species (Table 2-4). Other related compounds containing the CN^- anion, i.e. cyanate, thiocyanate, cyanogen chloride, are measured using separate methods outlined by "Standard Methods" (APHA-AWWA-WPCF 1981). Nitriles that do not hydrolyze in water and are not decomposed by mineral acids are determined by gas chromatography (ASTM 1982; DiGeronimo and Antoine 1976; Jensen et al. 1972; Sass et al. 1971; Steichen 1976).

Before any of these techniques (Table 2-4) can be applied, it is essential to treat the water sample for the presence of oxidizing agents and sulfides (ASTM 1982). If the sample cannot be analyzed immediately, it should be stabilized with NaOH to a pH of 12 or more and stored in a closed bottle, in a cool dark place (ASTM 1982). This will inhibit the escape of cyanide as HCN and the photolysis of iron cyanide complexes.

Free cyanide ($HCN + CN^-$), found predominantly as HCN at a pH of 8 and below, can be detected in samples of surface waters by the evolution and collection of hydrogen cyanide gas (Schneider and Freund 1962; Doudoroff et al. 1966; Nelson and Lysyj 1971; Broderius 1973, 1981; Fu-Sheng et al. 1981). It can also be measured in simple matrices using the method of Epstein (1947), as modified in APHA-AWWA-WPCF (1981), as well as the methods of Lambert et al. (1975), Wronski (1981) and Leung and Bartak (1981).

The method of Broderius (1981) for the determination of free cyanide as HCN with a detection limit of 1 $\mu g.L^{-1}$ HCN, involves the diffusion of HCN from an enclosed solution over a fixed time period into

Table 2-4. A comparison of the analytical methods used to measure cyanide.

Method of separation	Method of detection	Cyanide solutions yielding free cyanide	Cyanide solutions not yielding detectable free cyanide	Lower limit of detection ($\mu g.L^{-1}$)
Total cyanide by acid distillation (ASTM 1982) (APHA-AWWA-WPCF 1981)	a) Titration, or b) ion electrode for CN^-	free cyanide, metallocyanides (except cobalt III cyanide) some organocyanides	cobalt III cyanide some organocyanides cyanogen chloride cyanate, thiocyanate	a) 1000 b) 30 (ASTM 1982) 50 (APHA-AWWA-WPCF 1981)
	c) colorimetric	free cyanide, metallocyanides (except cobalt III cyanide) some organocyanides cyanogen chloride thiocyanate	cobalt III cyanide some organocyanides cyanate	c) 30 (ASTM 1982) 20 (APHA-AWWA-WPCF 1981)
Total cyanide by acid distillation under reduced pressure in the presence of zinc acetate (Roberts and Jackson 1971)	Colorimetric	free cyanide, metallocyanides (except cobalt III cyanide and iron II cyanide) some organocyanides cyanogen chloride	cobalt III cyanide iron cyanide some organocyanides cyanate, thiocyanate	2.0
Total cyanide by thin film acid distillation with UV irradiation and cyanide collection (Goulden et al. 1972) (Kelada et al. 1976)	Colorimetric	free cyanide, metallocyanides some organocyanides (e.g. acetonecyanohydrin and partial recovery of acrylonitrile) cyanogen chloride thiocyanate	some organocyanides (except partial recovery of acrylonitrile) cyanate	1.0 (Goulden et al. 1972) 0.25 (Kelada et al. 1976)
Cyanide amenable to chlorination after acid distillation (ASTM 1982) (APHA-AWWA-WPCF 1981)	a) Titration[a], or b) ion electrode for CN^-, or c) colorimetric	free cyanide, metallocyanides (except cobalt III cyanide and iron cyanides) some organocyanides cyanogen chloride	cobalt III cyanide iron II cyanide iron III cyanide some organocyanides thiocyanate	a) 1000
Weak acid dissociable cyanide (ASTM 1982)	a) Titration, or b) ion electrode for CN^-, or c) colorimetric	free cyanide dissociable metallocyanides e.g. Zn, Cd complexes	nondissociable metallocyanides, e.g. iron complexes (in absence of light), cobalt III cyanide, organocyanides, cyanate, thiocyanate	a) 1000 b) 30 c) 30
Thiocyanates and cyanides amenable to chlorination without distillation (APHA-AWWA-WPCF 1981)	Colorimetric	free cyanide, metallocyanides (except cobalt III cyanide and iron cyanides) cyanogen chloride thiocyanate	cobalt III cyanide iron cyanides cyanate	-
Simple cyanide by thin film acid distillation with cyanide collection (Goulden et al. 1972) (Kelada et al. 1976)	Colorimetric	free cyanide, metallocyanides (except cobalt III cyanide and iron cyanides) some organocyanides cyanogen chloride	cobalt III cyanide iron cyanides some organocyanides cyanate, thiocyanate	5.0 (Goulden et al. 1972) 0.25 (Kelada et al. 1976)
Free cyanide by diffusion and collection (Brodarius 1981)	Colorimetric	free cyanide dissociable metallocyanides eg. Zn, Cd complexes	nondissociable metallocyanides, eg. iron complexes (in absence of light), cobalt III cyanide, organocyanides, cyanate, thiocyanate	1.0
Free cyanide through oxidation and coupling in modified König reactions (Lambert et al. 1975)	Colorimetric	free cyanide	metallocyanides organocyanides cyanate	-

[a] only method recommended by ASTM (1982)

a dish of dilute NaOH suspended above the solution. The collected HCN is determined colorimetrically and the initial concentration calculated from calibration curves relating the quantity of HCN collected to the known concentration of HCN for standard cyanide solutions. Because the HCN diffuses into the NaOH solution, it is virtually unaffected by substances that interfere in the direct determination of free cyanide, including thiocyanate (Broderius 1982).

The total cyanide determination (APHA-AWWA-WPCF 1981; ASTM 1982) is accomplished in three stages. Initially a reflux distillation is carried out on the sample to break down all the metallocyanide complexes (except those of cobalt). Then the HCN so liberated is collected in an absorbing NaOH solution and determined by titration, colorimetry or specific ion electrode. The use of magnesium chloride as a catalyst helps to decompose the otherwise resistant iron cyanide complexes.

To determine the dissociable cyanide content of a sample another technique termed cyanide amenable to chlorination may be carried out (APHA-AWWA-WPCF 1981; ASTM 1982). Two duplicate samples are selected and one is chlorinated while the other is not. Both samples are then analyzed for total cyanide using acid distillation, the difference being cyanide amenable to chlorination (oxidizable cyanide); it consists of free cyanide, dissociable metallocyanides (e.g. dicyanocadmate II, tetracyanozincate II) and possibly some organocyanides. Strong metallocyanides (e.g. hexacyanoferrate II and III, hexacyanocobaltate III) are not measured. This method has a significant drawback in that some unidentified organic chemicals may be oxidized or alternately form breakdown products during chlorination, which could give higher results for cyanide after chlorination than before (APHA-AWWA-WPCF 1981). This in turn results in negative values for cyanide amenable to chlorination. To overcome this difficulty, the technique of weak acid dissociable cyanide (ASTM 1982) may be employed. This is essentially a modification of the total cyanide technique. The reflux distillation is performed in a slightly acidified sample rather than a strongly acidified one and a catalyst to decompose the iron cyanides is omitted. Moreover, zinc acetate is added to ensure that decomposition does not occur.

In addition to these procedures, several alternative methods of effluent analysis have been developed for the determination of cyanide and have generally been accepted by industry (E.A. 1979). Among these is a modification of the Roberts and Jackson (1971) procedure, which is essentially acid distillation under reduced pressure in the presence of zinc acetate. Recently, the conventional acid distillation technique was adapted for the analysis of mining effluents by Conn (1981). Hydrochloric acid and hydroxylamine hydrochloride were used as reagents and a small amount of solid cadmium chloride was added to the absorbing solution to precipitate sulfide. This procedure for measuring total cyanide recovers the CN from all the metallocyanides except the

cobalt complexes, is free from thiocyanate interference (up to 1000 $mg.L^{-1}$) and has produced acceptable results in an interlaboratory study. When tested in different effluent samples containing 30 $\mu g.L^{-1}$ total cyanides, the standard deviation was found to be 3 $\mu g.L^{-1}$. The modified distillation phase also improved upon the determination of cyanide amenable to chlorination, by minimizing thiocyanate interference.

The standard method for total cyanide analysis (APHA-AWWA-WPCF 1971) was modified by Goulden et al. (1972) to lower the limit of detection to 5 $\mu g.L^{-1}$ CN. This was achieved by recirculating the absorbing sodium hydroxide solution, thereby increasing the recovery of cyanide that was given off in the distillation phase. However, this method was found to be time-consuming because of the prolonged digestion in sulfuric acid required to decompose the complex cyanides (strong metallocyanides e.g. $[Co(CN)_6]^{-3}$, $[Fe(CN)_6]^{-3}$. To accelerate their breakdown, an ultraviolet irradiation pretreatment step was added and the apparatus was converted to permit sample analysis on a continuous, automated basis. In addition, phosphoric acid replaced sulfuric in the distillation step and the evolved HCN was collected using a sodium acetate solution, rather than sodium hydroxide. Using this modification, all metallocyanides (including those of cobalt) are decomposed.

With the UV pretreatment, total cyanide was determined. Without it, only simple cyanide (free cyanide and dissociable metallocyanides e.g. $Cd(CN)_2$, $[Zn(CN_4)^{-2}]$) was measured. The difference between total and simple cyanide was referred to as complex cyanide.

Ten samples of natural water and synthetic lake water could be analyzed per hour with a 1 $\mu g.L^{-1}$ CN detection limit. The coefficient of variation was estimated to be 1.2% at 40 $\mu g.L^{-1}$ CN.

Effluent analysis for free, complex and total cyanide was achieved by a modification of the Goulden et al. (1972) method, and has been approved by the U.S. EPA (Lordi et al. 1980). Details of the procedure are found in Kelada et al. (1976), who also report the percent recoveries of various cyanide species. Some of these data are reproduced in Table 2-5.

Table 2-5. The recovery of various cyanide species by a modification of the method of Goulden et al. (1972) (from Kelada et al. 1976).

Sample	CN Added ($\mu g.L^{-1}$) KCN	CN Added ($\mu g.L^{-1}$) Complex CN	Cyanide Measured: Simple CN ($\mu g.L^{-1}$)	Simple CN Recovery[α]	Cyanide Measured: Total CN ($\mu g.L^{-1}$)	Total CN Recovery[β]
Distilled water	0	0	0		0	
	10	25 CoIII	9.8	28.0	35.6	101.7
	25	25 CdII	48.7	97.4	50.0	100.1
Des Plaines River	0	0	2.1		11.7	
	10	10 FeIII	11.3	46.0	30.7	95.0
	25	10 NiII	35.8	96.3	45.4	96.3
Raw sewage	0	0	30.2		119.2	
	25	25 FeII	52.1	43.8	168.2	98.0
	25	25 MnIII	72.7	84.0	166.2	94.0
Treated sewage	0	0	4.3		25.2	
	25	25 CoIII	25.6	42.6	76.9	102.4
	25	25 CdII	40.9	73.2	73.0	94.6

[α] % recovery as simple cyanide =

$$100 \times \frac{\text{simple cyanide measured - simple cyanide in unspiked sample}}{\text{cyanide spiked}}$$

[β] % recovery as total cyanide =

$$100 \times \frac{\text{total cyanide measured - total cyanide in unspiked sample}}{\text{cyanide spiked}}$$

3.0 ENVIRONMENTAL DYNAMICS

3.1 INTRODUCTION

In a general sense, an assessment of the transport of cyanide-containing compounds through an aquatic ecosystem, including associated mechanisms and rates of transformation, would yield valuable scientific criteria which could be incorporated with observations of toxicological and ecological effects on resident biota. Unfortunately, reliable scientific information on the aquatic environmental dynamics of such compounds is practically nonexistent. The following discussion will therefore focus on the general principles of aquatic chemodynamics including as much information as is available for cyanide-containing compounds. It is hoped that such a treatment will provide the necessary stimulus for the generation of scientific information so that the cyanide cause-effect interrelations in the aquatic environment may be assessed.

When discussing the environmental fate of cyanides in water, one must consider the summation of all cyanide-containing compounds as well as the analytical methodology involved. From a toxicological point of view, free cyanide (HCN/CN^-) is considered to be the primary toxicant. However, from an ecological perspective, one must consider not only free cyanide but also those compounds that under given environmental circumstances have the potential for generating free cyanide. Moreover, some of these compounds may have toxicological or ecological impacts *per se*.

3.2 SOURCES

3.2.1 NATURAL

As discussed previously (see Section 2.6.7), many plants may synthesize cyanoglycosides which upon decomposition may lead to the formation of free cyanide. In addition, numerous microorganisms have the ability to produce free cyanide either as their own metabolic product or through the decomposition of cyanogenic compounds.

Among the fungi, the snow mold fungus (an unidentified basidiomycete) is a prolific producer of HCN in laboratory liquid cultures, glycine being used as a precursor (Knowles 1976). In bacteria, cyanide production has been observed in *Chromobacterium violaceum* and certain species of *Pseudomonas*. The conditions of cyanogenesis by these organisms are more strict than for simple cell growth. Glycine is the main metabolic precursor but cyanogenesis is enhanced by methionine, even though this amino acid is not a precursor of cyanide. In *Pseudomonas* the presence of oxygen is essential to the production of HCN, and specific concentrations of iron and phosphate are required for

optimal cyanogenesis (Meganathan and Castric 1977; Castric 1977; Rodgers and Knowles 1978; Castric et al. 1979). Many photosynthetic microorganisms among the green and blue-green algae can produce free cyanide in the process of nitrate metabolism. Chlorella does not produce appreciable amounts of HCN but Anacystis nidulans, a microscopic blue-green algae, used histidine to release substantial amounts of HCN, raising the concentration of a culture medium to 0.5 $mg.L^{-1}$ in three hours, (Pistorius et al. 1979). Another biochemical source of free cyanide is the decomposition of cyanoglycosides of higher plant origin by microorganisms. There is, however, little known of the effects of low sublethal concentrations of free cyanide from biochemical origins, and the subject deserves much more attention.

3.2.2 ANTHROPOGENIC

The release to the aquatic environment of compounds containing the cyanide group has traditionally been associated with discharges from point sources resulting from certain industrial activities as well as from nonpoint sources resulting from the application on land and water of chemicals containing cyanide compounds. Detectable levels of cyanide in receiving waters are considered to be the result of improper management practices and may occur as a result of improper storage, handling and disposal of wastes (Towill et al. 1978).

Although the destruction of cyanide in industrial wastewaters can be accomplished by a variety of procedures, alkaline chlorination is one of the most widely used methods (APHA-AWWA-WPCF 1981). The introduction of chlorine produces cyanogen chloride (CNCl), a highly toxic gas of limited solubility:

$$XCN + Cl_2 \rightarrow CNCl + XCl \qquad (3\text{-}1)$$

where $X = K^+$, Na^+ etc.

At an alkaline pH, cyanogen chloride hydrolyzes to the cyanate ion:

$$CNCl + 2NaOH \rightarrow NaCNO + NaCl + H_2O \qquad (3\text{-}2)$$

$$NaCNO \rightarrow Na^+ + CNO^- \qquad (3\text{-}3)$$

Since the cyanate ion is approximately three orders of magnitude less toxic than HCN and because it does not release CN^- upon degradation (Resnick et al. 1958), it is commonly released to the environment at this stage. If free chlorine is present, CNO^- can be further oxidized to CO_2 and N_2:

$$2NaCNO + 4NaOH + 3Cl_2 \rightarrow 6NaCl + 2CO_2 + N_2 + 2H_2O \qquad (3\text{-}4)$$

Other methods for treating cyanide wastes include lagooning (natural degradation), ozonation, acidification/volatilization/reneutralization, ion exchange, activated carbon absorption and electrolytic processes (Ingles and Scott 1981). Cyanide can also be

converted to thiocyanate and ferrocyanide. Dodge and Reams (1949) found that cyanide reacted with polysulfides in solution yielding thiocyanate. However, if the thiocyanate contacted chlorine or other oxidizing agent at low pH, HCN was released.

Conversion of cyanide and cyanide complexes to ferrocyanide was one of the earliest methods of treatment but is not recommended any longer since ferrocyanide may release the CN^- ion in the presence of ultraviolet light.

A major anthropogenic source of cyanide in Canada is the gold mining and milling industry which commonly discharges cyanide in the $mg.L^{-1}$ range. A survey of cyanide concentrations in the final effluents of six Canadian gold mills revealed 0.3 to 26.5 $mg.L^{-1}$ total cyanide (Scott and Ingles 1981). Cyanides are also present in the effluents of petroleum refineries (Côté 1976; Kunz et al. 1978). In addition, numerous metal finishing factories and steel mills are known to release cyanide in their liquid effluents. Although reliable data are not readily available for Canada, examples of ranges of total cyanide concentrations in effluents of some industrial activities are illustrated in Table 3-1.

The release of cyanide-containing material to receiving waters can result in significant impacts on resident biota. For example, a leak of effluent containing cyanide into Ladner Creek, a tributary of the Coquihalla River, occurred from a tailings pond at the Carolyn Mines, near Hope, British Columbia. Approximately 20,000 steelhead smolts (Salmo gairdneri) were killed. The concentration of cyanide (total?) immediately downstream of the mine was found to be 8 $mg.L^{-1}$ (Waldichuk 1982).

Material containing cyanide compounds disposed of on land may lead to elevated levels of cyanide in underlying and groundwater (cf. Army Engineer Waterways Experiment Station Report 1978). The environmental significance of cyanide-containing compounds leaching from land-disposal sites has not been fully examined.

3.3 LEVELS

Data on the distribution of various cyanide compounds within that aquatic environment are limited. Most surveys conducted to date have relied upon the measurement of some form of total cyanide without having adequately defined the chemical species measured, the interferences in the analysis that may have been encountered, or the detection limits of the particular technique. Although some monitoring has been performed, national baseline (background) levels of cyanide in natural water apparently cannot yet be established, in part because of the different and sometimes improper analytical methods employed by the different investigators and to the different methods of recording and interpreting such data.

Table 3-1. Exemplary cyanide concentrations in effluents of certain industries[α].

Type of industry	Levels of total cyanides observed ($mg.L^{-1}$)
Steel mill (weak ammonia liquor)	30-60
Oil refinery	2-4
Electroplating shops[β]	3 (0.1 oxidizable cyanide)

[α] Table 3-1 supplied by Dr. H.S. Samant, EPS, Halifax.

[β] Electrolyte baths may contain 2-3% total cyanide. Electroplating shops operate on batch mode and are not expected to have continuous effluents. The baths are not to be discharged to the environment without treatment to meet the guidelines/objectives.

Cyanides have been identified in fresh waters of rural and wilderness areas in both Canada (P.P.W.B. 1978) and Germany (Krutz 1981). The occurrence of cyanide has been related to the size of the watershed and seasonal runoff (Krutz 1981).

A survey carried out from 1974 to 1977 in Canada (P.P.W.B 1978) examined 50 physical and chemical parameters, including total cyanide, in eleven rivers located in the western and central parts of the country. Levels of total cyanide were often recorded as high as 30-60 $\mu g.L^{-1}$. The concentrations of cyanide differed considerably but seemed related to runoff. In small rivers with a discharge rate of 28 to 140 $m^3.s^{-1}$ and medium size rivers (140 to 710 $m^3.s^{-1}$), peaks of cyanide were more frequent in fall and winter during minimal runoff. In larger rivers (430 to 2100 $m^3.s^{-1}$) the peak cyanide levels occurred during and/or after the May-June runoff.

Krutz (1981), using a modification of the analytical technique of Ludzack et al. (1954) (detection limit of 0.1 $\mu g.L^{-1}$), reported mean annual levels of about 3 $\mu g.L^{-1}$ total cyanide in rural watersheds compared with 20 $\mu g.L^{-1}$ in industrial areas of Germany. In small watersheds covered with grasslands and forests without settlement, total cyanide concentrations averaged between 0.7 and 2 $\mu g.L^{-1}$ with peak concentrations of 5 $\mu g.L^{-1}$ occurring in fall and winter. In larger rivers, cyanide levels were low in winter but peaked in summer.

It is important to remember that the levels of cyanide measured in these field studies are expressed as total cyanide. Not all of the various contributing cyanide species may be toxicologically significant. For example, thiocyanate, which is relatively nontoxic may be included (depending on the technique used) in the total cyanide determination. Selective determinations of free cyanide which are properly used are needed to record the actual levels of free cyanide in surface waters.

3.4 FATE IN THE AQUATIC ENVIRONMENT

Most of the currently held opinions regarding the fate of cyanide-containing compounds in the aquatic environment are based on investigations on the treatment of complex industrial wastes containing cyanides at levels in the $mg.L^{-1}$ range, usually in association with a multitude of other substances. Concentrations in wastewaters were found to be lowered by such mechanisms as chemical oxidation, irradiation, biodegradation, volatilization, photolysis, dissociation and precipitation. The relevant literature has been reviewed recently (Miller and Villaume 1978; Towill et al. 1978; E.A. 1979; IEC 1979).

Based upon the information obtained from effluent treatment and from a few relatively simple laboratory experiments using very high cyanide concentrations, it has been generally assumed that cyanide has a

short residence time in the aquatic environment. However, it is often difficult and misleading to extrapolate such information using relatively high cyanide concentrations to the natural environment because of such modifying factors as temperature, pH, sunlight, turbidity and biological activity. From the relatively few studies involving *in situ* measurements in certain segments of the aquatic environment one cannot assume that various cyanide-containing compounds have a short residence time in natural waters.

3.4.1 FREE CYANIDE

Hydrogen cyanide is volatile, having a vapor pressure of 53.1 kPa at 10.2°C, and will be in equilibrium with atmospheric HCN approximately according to Henry's Law (Milne 1950). The distribution coefficient between air and water phases is approximately 3×10^{-3} mg $HCN.L^{-1}$ gas/mg $HCN.L^{-1}$ water (Schneider and Freund 1962). Volatilization, especially with turbulence, is expected to be a significant means of free cyanide removal from concentrated effluent (IEC 1979); however, it may be a rate-determining step at low concentrations associated with its loss from natural waters.

Chemical oxidation of free cyanide occurs via stepwise reactions with the conversion of free cyanide to cyanogen and cyanate. Cyanate is then hydrolyzed to carbonate and ammonia. Although chemical oxidation of free cyanide is a commonly-used treatment method (White 1972; Kunz *et al.* 1978), reliable data on the chemical oxidation of free cyanide in natural water is not available.

Data on the absorption of free cyanide to either suspended particles or bottom sediments in natural ecosystems are not available. Murrmann and Koutz (1972) demonstrated that the cyanide ion is not strongly adsorbed and retained in soils. The cyanide salts that were tested moved only a short distance through the soil before being either biologically converted under aerobic conditions to nitrates or fixed by trace metals through complex formation.

Numerous microorganisms have been identified which are capable, under certain conditions, of degrading free cyanide (Towill *et al.* 1978). Cyanide may be removed from waste streams by biological treatment (Kunz *et al.* 1978), although acclimation of mixed cultures is usually necessary. Microbial degradation of free cyanide in receiving waters has not been adequately studied.

3.4.2 COMPLEX CYANIDES

Molecular HCN, formed in the presence of certain complex cyanides, will be subjected to the same removal mechanisms as discussed above. Little information is available on the fate of complex cyanides in natural waters. The role of precipitation from solution and

adsorption to solids and bottom sediments in the removal of complex cyanides from the water column has not been adequately examined.

Many metallic cyanide complex ions are stable in aqueous solution in the absence of ultraviolet and visible light; in the presence of sunlight, the photodecomposition of complexes may occur. The photodecomposition of hexacyanoferrates has been extensively studied by Broderius and Smith (1980) (see Section 2.5.4) and has been found to vary with water turbidity and color, presence of other light-absorbing materials, geographic location, angle of incidence of radiation, time of day, season, extent of cloud cover and degree of water mixing.

Reviews on the biological removal of cyanides from industrial waste waters are available (cf. Howe 1965; Raef et al. 1977a, 1977b); however, most studies have dealt with free cyanide or simple cyanides and not complex metallocyanides per se.

3.4.3 FIELD STUDIES

Although cyanides may be rapidly removed by in-plant treatment (with the exception of hexacyanoferrate II and III and hexacyanocobaltate III unless irradiated) long residence times are required in treatment lagoons. For example, Howe (1965) reported that a residence time of 180 days was required in a lagoon having a surface area of 2.8 hectares to lower total cyanide concentrations from 170-200 $mg.L^{-1}$ to 26 $mg.L^{-1}$. A further 240 days was required to lower the cyanide concentration to 3 $mg.L^{-1}$ in a second lagoon of 2.4 hectares. The removal of cyanides from gold mine effluents was investigated at a northern Ontario mine site. The total cyanide concentration in the barren bleed holding pond decreased from 68.7 $mg.L^{-1}$ to 0.08 $mg.L^{-1}$ (99.9% reduction) over a period from April to September and appeared to be related to an increase in water temperature. Volatilization and oxidation of free cyanide to cyanate were thought to be the primary means of cyanide removal (Schmidt et al. 1982).

Several investigations concerning the release from point sources and aquatic transport of cyanides have been reported. Chester Engineers Ltd (1977, cited in E.A. 1979) estimated the rate of removal of cyanide in a river to be 3.1 $\mu g.L^{-1}.h^{-1}$ at a distance of 5.2 km from the source; the rate of removal decreased to 0.3 $\mu g.L^{-1}.h^{-1}$ 17.7 km from the source. This finding was interpreted by the researchers to mean that removal may have been limited by HCN volatilization. The concentration of cyanide in the immediate vicinity of the source was not reported in this study.

Studies on the Flat River, Northwest Territories, have demonstrated that total cyanide ranged from 0.05-0.25 $mg.L^{-1}$ near a mine site, but returned to background levels 5 to 9 km from the source (Moore 1980). The author considered that seasonal variation in cyanide

concentrations was not substantial, although data were not presented. Water temperature ranged from 0°C in winter to 8-10.5°C in summer during the course of this study.

Monitoring studies have been conducted on the Illinois River where the main sources of cyanides are municipal waste treatment plants at Chicago (Lordi et al. 1980). The treatment plants serve several large steel mills that have coking operations and over 400 electroplating facilities.

The concentration of free cyanide (i.e. determined as "total" minus "complex" cyanide, Section 2.7) decreased in the river from approximately 5 $\mu g.L^{-1}$ at Chicago to 1 $\mu g.L^{-1}$ at a point 133 km downstream. The free cyanide concentration remained at this level for at least another 120 km. The total cyanide concentration (including hexacyanoferrate II and III complexes) decreased from 38 $\mu g.L^{-1}$ at Chicago to 19 $\mu g.L^{-1}$ over a distance of only 20 km. For the next 150 km, total cyanide varied between 13 and 16 $\mu g.L^{-1}$. Conceivably, photolysis of hexacyanoferrate complexes was partially responsible for the initial decrease in total cyanide and the presence of free cyanide. An equilibrium may have been established between photolysis and volatilization which kept HCN concentrations to a minimum.

The aquatic transport and transformation of some cyanide-containing organic compounds have been examined. For example, monitoring in the vicinity of plastics manufacturing sites in the United States revealed that acrylonitrile concentrations in surface waters ranged from not detectable to 4.3 $mg.L^{-1}$ (acrylonitrile) (Going et al. 1978). However, the fate of acrylonitrile released to the aquatic environment was not further investigated in this study. The capacity for biodegradaton of various nitriles in the Ohio River was determined by Ludzack et al. (1958). The resident microfauna in the water samples were briefly preexposed to simulate acclimation to the presence of the contaminants. Then a high concentration of a nitrile was added to each water sample and changes in the concentration were measured over an extended time period. At room temperature acrylonitrile was degraded at a rate comparable to those of other nitriles but at a temperature simulating winter conditions (Figure 3-1), it was the slowest to be degraded (more than 40 days). A lower temperature resulted in a longer induction period for acrylonitrile (approximately 22 days were required before 10% of the acrylonitrile was degraded).

Without preexposure and acclimation however, oxydipropionitrile was most resistant to degradation at room temperature, requiring 125 days for 60% degradation of 12.5 $mg.L^{-1}$. Similar results for oxydipropionitrile were reported by Cherry et al. (1956). Benzonitrile was apparently degraded most rapidly at both temperatures following acclimation of the microfauna.

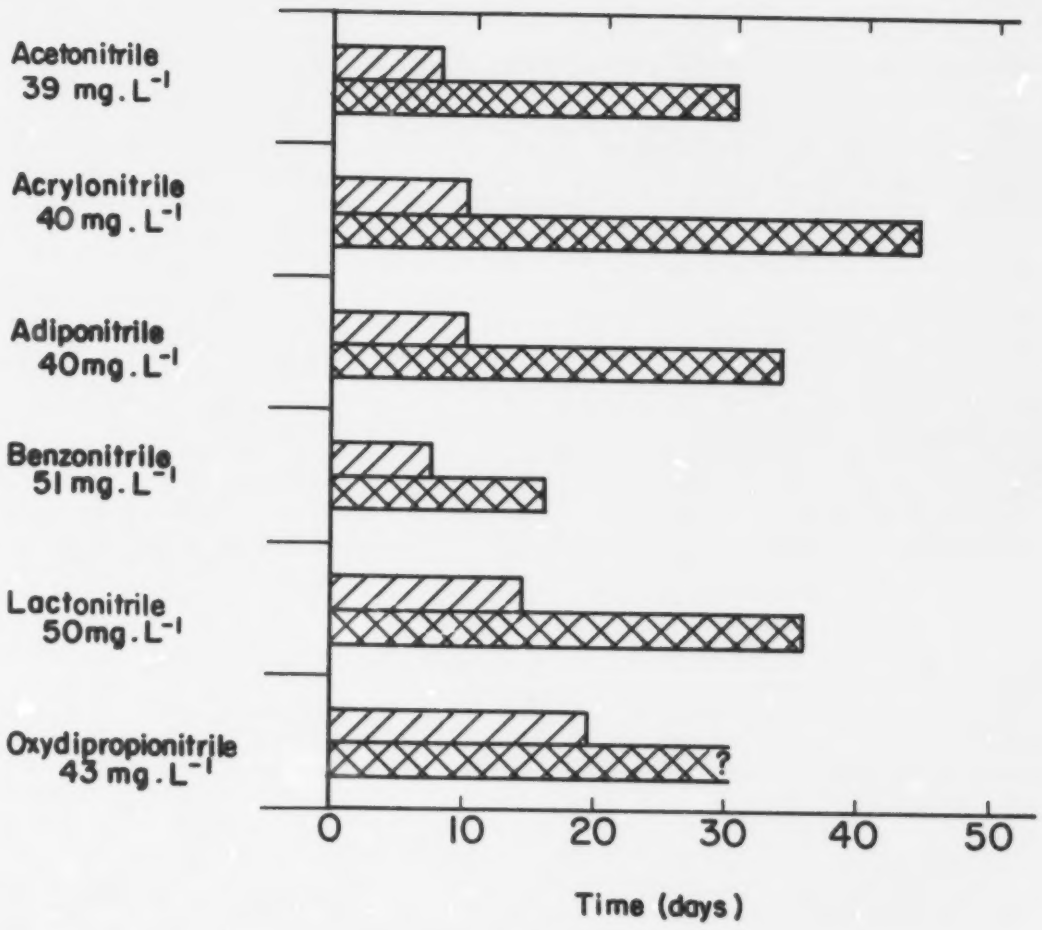


Figure 3-1. Effect of temperature on nitrile degradation[α]. (from Ludzack et al. 1958).

[α] Carbon dioxide emission was used to indicate nitrile degradation as reliable chemical analysis for nitriles did not exist at the time of this study.

20°C
5°C

10-60% of theoretical CO_2

4.0 TOXICOLOGY

4.1 INTRODUCTION

The toxicology of free cyanide ($HCN + CN^-$) has been investigated in more detail than that of other cyanides and is the main focus of this section. The discussion covers its mode of action together with lethal and sublethal exposure regimes, emphasizing the physiological responses of ecological significance. Although most of the studies were carried out on fishes, the available information concerning other organisms is also reported.

4.2 FREE CYANIDE

4.2.1 MODES OF ACTION

The mode of action of cyanide has generally been attributed to the inhibition of the enzyme cytochrome oxidase. There are however several other enzyme-mediated reactions affected by cyanide. The various aspects of cyanide's mode of action, metabolism and detoxification are presented in the comprehensive review by Vennesland et al. (1981).

4.2.1.1 Cytochrome Oxidase Inhibition

The acute mode of action of HCN is limited to binding those porphyrins which contain iron III. Following deprotonation, the cyanide ion forms a complex with the central porphyrin iron according to the reaction (Chance 1949):

$$FeOH + HCN \rightleftarrows FeCN + H_2O \qquad (4\text{-}1)$$

The primary toxic effect is considered to be the inhibition of the porphyrin subunit (cytochrome a_3) of the cytochrome oxidase, with consequent blockage of both electron transport and associated ATP production (oxidative phosphorylation). Cyanide thus deprives the cell of ATP energy potential.

Other molecules containing the iron III porphyrin subunit include oxidases, catalases, and methemoglobin. (Methemoglobin is produced by the oxidation of the iron II porphyrin group of hemoglobin.) These are susceptible to cyanide inactivation by the same mechanism as that for cytochrome oxidase (equation 4-1), but this inactivation is apparently less crucial to the survival of an organism. Dissociation constants of FeCN in the porphyrins are 3.6×10^{-6} M for catalase and 5×10^{-7} M for cytochrome oxidase (Chance 1949; Stannard and Horecker 1948), indicating that inhibition is reversible.

The inhibition of cytochrome oxidase is noncompetitive and, hence, is independent of the relative proportions of toxicant and substrate with which the enzyme reacts (Giese 1973). Thus, the acute toxicity of cyanide will be determined almost completely by the concentration of cyanide (in the mitochondrial matrix) surrounding the structure on which cytochrome oxidase is thought to be located (oxysomes of the mitochondrial cristae). Inhibition is likely to be minimal until concentrations of HCN reach approximately 5×10^{-7} M (from the dissociation constant for FeCN) in the mitochondrial matrix. Differences in sensitivity to cyanide may occur between mitochondria of different animal species, and many plant mitochondria contain electron transport chains which are insensitive to cyanide (Moreland 1980). It is interesting that while the concentration of cytochrome oxidase in fish mitochondria apparently does not vary with ambient temperature, the activity of the enzyme is greater in mitochondria from cold-associated individuals (Hochachka 1967). Therefore, one would expect fish to be more sensitive to cyanide when acclimated to lower temperatures (see Section 4.2.2.2).

When cyanide stops the production of ATP associated with the electron transport chain, another metabolic pathway is activated (Pasteur effect) and ATP is synthesized via the degradation of glucose to lactic acid, or an equivalent end product (Embden-Meyerhof pathway). Degradation of glucose, commonly referred to as glycolysis, does not utilize oxygen but does not produce sufficient ATP to sustain organisms that are relatively active. Most sessile invertebrates and many microorganisms, however, are capable of continued anaerobic metabolism via glycolysis. Such organisms are expected to be resistant to acute cyanide exposure.

Long-term lethal exposure and sublethal exposure to HCN may be first perceived as the inactivation of a certain fraction of cytochrome oxidase molecules. Accordingly, ATP production is reduced in proportion to the degree of enzyme inactivation, but is not completely arrested. An organism may compensate by becoming less active and thereby decreasing its demand for ATP. Thus, larval Atlantic salmon (Salmo salar), which were observed to be substantially less active than controls, may have accommodated to the presence of cyanide (Leduc 1978). Alternatively, an organism may replenish all or part of the ATP via glycolysis. This concurs with reports that organisms, whether protozoans or mammals, rapidly increase lactic acid concentrations in their tissues upon exposure to cyanide (Lochner et al. 1959). In exposed fish, an inverse relationship has been demonstrated between the activity of a glycolytic enzyme (aldolase) and the activity of cytochrome oxidase (Leduc 1966).

The glucose molecules required for continued ATP production via glycolysis may be obtained from food (glucogenesis) and from body reserves of fat and protein (gluconeogenesis). Glycogen reserves in the liver are consumed initially as an immediate source of glucose.

Apparently, the glycogen reserves are replaced under chronic cyanide exposure conditions by a transformation of fat and protein similar to that in migrating salmon (Hoar 1966). This hypothesis tends to be supported by the results of Kovacs (1979) who exposed rainbow trout (Salmo gairdneri) to 15 $\mu g.L^{-1}$ HCN for a period of 20 days. Liver glycogen was initially consumed while lactic acid increased. After 10 days' exposure, these levels returned to normal. Presumably the lactic acid was reconverted to glucose in the liver; a plausible pathway that was proposed by Hochachka and Somero (1973). It is interesting to note that the changes in liver glycogen occurred in fish acclimated to 6°C and 12°C, but not 18°C. The influence of temperature may be interpreted in terms of higher cytochrome oxidase activity in cold-acclimated fish, in addition to other possible biochemical interactions.

4.2.1.2 Succinate Dehydrogenase Inhibition

The mitochondrial electron transport system may also be seriously disturbed by cyanide through the inhibition of succinate dehydrogenase, which is a nonheme flavin-containing iron-sulfur protein which passes electrons to the cytochrome system. The peculiarity of this enzyme is its iron sulfur linkages which are of the persulfide or sulfane type (-S-S), favoring a "labile sulfur" condition that is essential for activity. Cyanide is a strong thiophile reacting with the "labile" sulfur thus breaking the persulfide bond. The K value for the reaction:

$$R\text{-}S\text{-}S\text{-}R + HCN \rightarrow RSH + RSCN \qquad (4\text{-}2)$$

is so high (Westley 1981) that the reaction is irreversible and would render the enzyme incapable of hydrogen transfer.

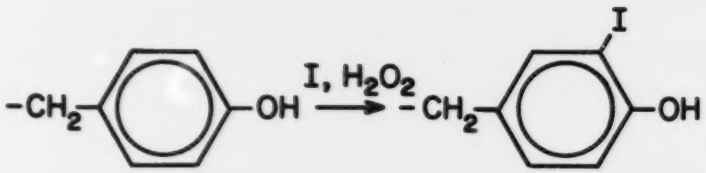
4.2.1.3 Hydroperoxidase Inhibition

Hydroperoxidases consist of two classes of enzymes, catalases and peroxidases, both of which utilize hydrogen peroxide as a substrate. The ferro-heme group is strongly inhibited by cyanide, although the physiological significance at sublethal levels remains obscure. The main function of catalase is to decompose hydrogen peroxide, which is a powerful oxidant:

$$H_2O_2 + AH_2 \rightarrow 2H_2O + A \qquad (4\text{-}3)$$

There are various sources of H_2O_2, namely autoxidizable substances (AH_2) and amino oxidases (flavin-containing proteins). One tissue that could be seriously affected by the inhibition of catalase by cyanide is the blood, where the formation of H_2O_2 would lead to the oxidation of hemoglobin (Fe^{2+}) into methemoglobin (Fe^{3+}), thus reducing the oxygen carrying capacity of the organism.

Reduced peroxidase activity caused by cyanide could adversely affect the synthesis of the thyroid hormone since the iodination of tyrosine takes place in the presence of oxygen and peroxidase (Metzler 1977).



(4-4)

4.2.1.4 Vitamin B_{12} Deficiency

During chronic cyanide exposure the precursors of Vitamin B_{12} may react with cyanide, thereby reducing the amount of available B_{12}. The cobalamines react strongly with cyanide and can also assist in cyanide detoxication. However, the monopolization by cyanide of precursors such as hydroxymethyl- and 5-deoxyadenosyl-cobalamines into cyanocobalamines will deplete the pool of physiologically active forms of B_{12}.

One of the main roles of B_{12} is to act as a cofactor in the methylation of homocysteine to methionine which is later transformed into a universal methyl group donor. Transmethylation reactions are quantitatively essential to the metabolism of all cells.

Vitamin B_{12} deficiency is associated with such disorders as pernicious anemia, blocked mitosis and DNA synthesis and demyelination of the central nervous system, causing ataxia and psychotic ailments. Neuropathological changes and lower vitamin B_{12} levels have been observed in rats subjected to chronic cyanide intoxication (Smith et al. 1963).

4.2.1.5 Thiocyanate Formation and Goitrogenicity

Widely distributed in nature is the enzyme rhodanese (thiosulfate sulfur transferase) which detoxifies HCN by conversion to SCN^-. This enzyme is found in animals, plants and prokaryotes and its physicochemical properties have been reviewed by Westley (1973, 1977).

Rhodanese, located in the mitochondrial matrix, plays a key role in the sulfur metabolism of the cell and its role in the detoxication of cyanide is only incidental. More specifically, rhodanese catalyzes the transfer of sulfane-sulfur atoms to thiophilic groups:

$$(RSO_xS) + \underset{\text{acceptor}}{\text{Thiophilic}} \rightleftarrows RSO_x + \underset{\text{anion}}{\text{Thiolated}} \qquad (4\text{-}5)$$

The reaction involves a sulfur-substituted intermediate which will react strongly with a sulfur nucleophile such as CN^- to form the less toxic thiocyanate ion:

$$SSO_3^{-2} + \text{Enzyme} \rightleftarrows SO_3 + \text{Enzyme-S} \qquad (4\text{-}6)$$

$$\text{Enzyme-S} + CN^- \rightarrow SCN^- + \text{Enzyme} \qquad (4\text{-}7)$$

The capacity of this mechanism to detoxify cyanide was demonstrated using sodium thiocyanate, which is a sulfane-sulfate donor. Thiosulfate administration was found to increase the resistance of fish to cyanide (Achard and Binet 1934).

However, the role of rhodanese in detoxification may lead to complications during chronic cyanide exposure. First of all, cyanide may be regenerated through the oxidation of thiocyanate (Westley 1981):

$$SCN^- + 3H_2O_2 \rightarrow CN^- + SO_4^{+2} + 2H_3O^+ \qquad (4\text{-}8)$$

The enzymatic decomposition of thiocyanate into cyanide by a thiocyanate oxidase has been found in mammals, but only in erythrocytes. Accordingly, the enzyme may ensure a constant level of free cyanide in the blood. This has been interpreted to mean that HCN, in minute quantities, might be an essential metabolite (Smith et al. 1963). Under conditions of chronic exposure, the enzyme may contribute to toxicity by increasing blood cyanide levels.

Thiocyanate produced by rhodanese may also produce adverse effects in the thyroid gland (Yamada et al. 1974). Hence, chronic assimilation of cyanide by mammals has been related to increased levels of plasma thiocyanate and the occurrence of goiter. Thyroid abnormalities are perhaps more likely to occur in aquatic organisms exposed to both thiocyanate and cyanide as constituents of the same industrial effluents. The development of goiter in fish exposed to cyanide has yet to be demonstrated, although abnormal thyroid conditions in fish of the Great Lakes have been associated with environmental contaminants (Leatherland and Sonstegard 1978). The potent goitrogenicity of thiocyanate-like compounds (e.g. thiourea) in fish is consistently associated with growth inhibition in addition to numerous other detrimental effects (Donaldson et al. 1979). However, slight antithyroid activity may be associated with growth enhancement in mammals (Tata 1974) and this point will be discussed in more detail in Section 4.2.3.3. Normal thyroid function in fish is associated with physiological adaptations to the environment, including temperature acclimation and osmoregulation during migratory fresh-to-saltwater (or salt-to-freshwater) transitions (Etkin and Giona 1974).

Basically, two important physiological functions may be affected by cyanide poisoning: 1) the respiratory chain by HCN and 2) the thyroid gland by SCN^-. The relative proportion of HCN to SCN^- will, however, be a function of rhodanese activity. This enzyme is present in such large quantities in tissues that the limiting factor is believed to be mobilization and supply of sulfur. The amount of sulfur in the diet can therefore be a determining factor.

With regard to the effects of the thyroid gland, it is known that the symptoms of hypothyroidism can be overcome by the addition of iodine to the diet. This has long been verified in rabbits fed with cyanoglycosides. Hence, varying amounts of iodine intake by an organism will influence the overall response to SCN^-.

A third environmental chemical is likely to interfere with cyanide poisoning. Nitrites often occur in water loaded with organic matter but low in oxygen, or in association with ammonia-containing wastes. Once in the blood, nitrites oxidize hemoglobin into methemoglobin which will react strongly with HCN to form cyanomethemoglobin. Moderate amounts of methemoglobin reduce the blood oxygen carrying capacity but protect the respiratory chain from the toxic action of cyanide. This technique of rapid induction of methemoglobin is applied to humans in cases of acute poisoning: the CN MeHB is later metabolized by rhodanese with the release of SCN^-.

Different intensities of hypothyroidism and hypoxia will occur depending on the occurrence of sulfur, iodide and nitrite present in the food and/or the water. The intervening responses to different combinations of these chemicals have not yet been measured but the scheme presented in Figure 4-1 suggests the complexity of the various routes of cyanide poisoning. Depending on the availability of sulfur (-S-S), iodide (I^-) and nitrite (NO_2^-), cyanide will take different "circuits" and have different biological effects. High sulfur and low iodide will lead to the formation of SCN^- and possibly thyroid effects, whereas low sulfur and medium iodide would allow SCN^- and HCN to exist with intermediate effects on respiration and thyroid function. High iodide levels can counteract the effects of high SCN^- levels. The tentative role of nitrite in the scheme is the binding of cyanide and the release of SCN^-. This mechanism could be of high protective value for the respiratory chain during short pulses of cyanide.

4.2.2 LETHAL EXPOSURES

4.2.2.1 Field Observations

There have been numerous reports of massive fish kills due to cyanide. These incidents occur mainly following accidental spills from storage reservoirs and overturned railway tank cars containing concentrated cyanide solutions. In addition, acutely lethal conditions have resulted from the direct release of cyanide-containing wastewaters. Cyanide has also been intentionally used as a fish poison to collect fish from rearing ponds (Bridges 1958; Lewis and Tarrant 1960; Prewitt 1970) and to restore lakes (Leduc et al. 1973).

In these situations, biological response is usually immediate. Fish become agitated within minutes, rise to the surface, gulp for air, swim rapidly in circles at the surface and enter into convulsions before

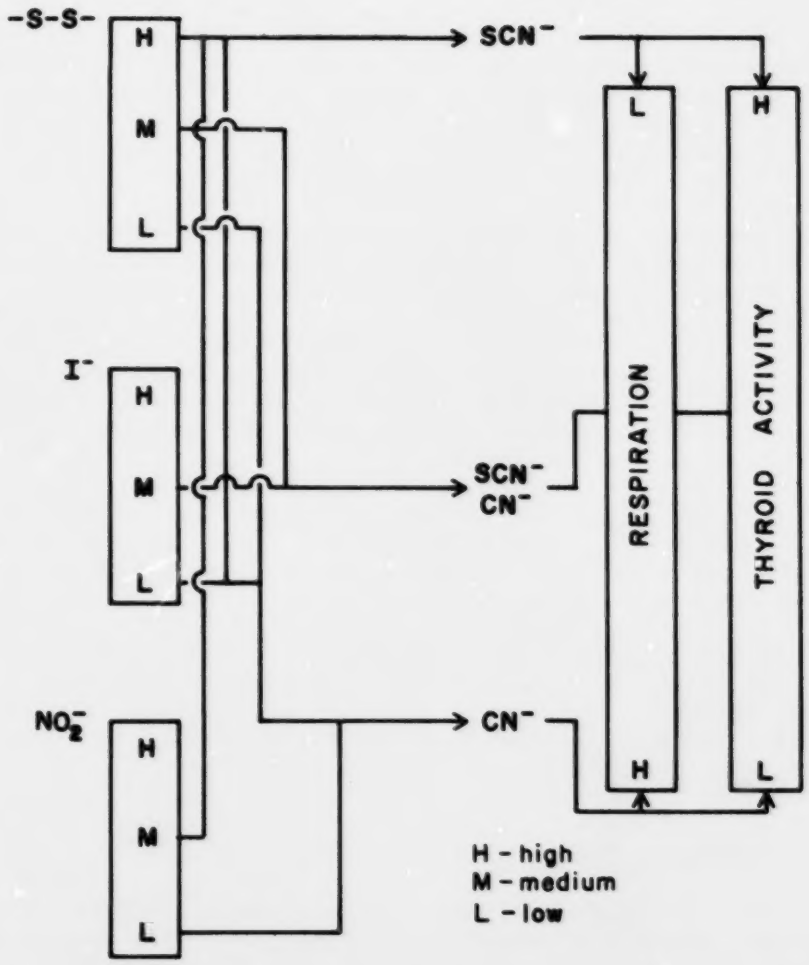


Figure 4-1. Various pharmacodynamic models of cyanide in the presence of different concentrations of sulfur, iodide and nitrite.

dying. In small, cold oligotrophic lakes treated with 1 $mg.L^{-1}$ NaCN, the toxicity disappeared after 40 days (Leduc et al. 1973). In warm shallow ponds the toxicity apparently ceased within 4 days after the application of 1 $mg.L^{-1}$ NaCN (Bridges 1958). However, many accidental spills have involved complex cyanides, which have not been adequately studied with respect to environmental residence times and time-mortality relationships.

The primary mode of action of cyanide is fast but reversible. Fish overcome by cyanide, if removed soon enough and transferred to clean water, will revive with no apparent ill effects (Bridges 1958). Fish retrieved, dead or alive, during or after cyanide exposure may be consumed by man because the amount in the tissue is too small to be of any harm ($< 1\ \mu g.g^{-1}$ of fish) (Kariya et al. 1967).

The severity of a fish kill following an accidental spill will vary with location and with the species involved. Field observations by Leduc et al. (1973) revealed marked differences in sensitivity. Brook trout (Salvelinus fontinalis) responded to the poison within minutes, died rapidly (they were too sensitive to be revived in clean water), and most of them sank to the bottom. Therefore, it would be difficult to observe brook trout mortality following an accidental spill. In contrast, other species float at the water surface even after death. Such species as yellow perch (Perca flavescens), white suckers (Catastomus commersoni), pumpkinseed sunfish (Lepomis gibbosus), rock bass (Ambloplites rupestris), burbot (Lota lota), and various species of minnows, (Cyprinidae), reacted slowly and could be revived (Bridges 1958). The most resistant species appeared to be the brown bullhead, (Ictalurus nebulosus).

Other organisms (e.g. frogs, salamanders, tadpoles, mayfly larvae) are susceptible to cyanide. Despite mortality among these organisms, lakes treated with cyanide may rapidly recover their invertebrate fauna as inferred from the growth of fish in newly-stocked lakes (Leduc et al. 1973).

4.2.2.2 Laboratory Observations

The literature pertaining to laboratory toxicity studies has been reviewed recently (Doudoroff 1976, 1980) and the following sections are intended as a brief presentation with an emphasis upon newly published work. The acute toxicity of free cyanide to various species of juvenile freshwater fishes and freshwater invertebrates is given in Table 4-1 and Table 4-2 respectively. Generally, concentrations greater than 100 $\mu g.L^{-1}$ as HCN can be expected to kill sensitive fish species in either freshwater or marine environments (Doudoroff 1976; Cardwell et al. 1976).

Although the data concerning the lethal toxicity of cyanide to invertebrates is limited, it appears that invertebrates are much less

Table 4-1. Acute toxicity of cyanide[a] to different species of juvenile freshwater fish at various temperatures and at or near saturation levels of dissolved oxygen.

Species	Temperature (°C)	96-h LC_{50} ($\mu g.L^{-1}$)	Lethal Threshold Concentration ($\mu g.L^{-1}$)	Reference
rainbow trout	6.0	28	-	Kovacs (1979) and Kovacs and Leduc (1982a)
Salmo gairdneri	10.0	57.2	-	Smith et al. (1978)
	12.0	42	-	Kovacs (1979) and Kovacs and Leduc (1982a)
	18.0	68	-	Kovacs (1979) and Kovacs and Leduc (1982a)
brook trout	6.9	75.4	73.9	Smith et al. (1978)
Salvelinus fontinalis	10.0	88.4	83.8	
	13.0	98.5	96.5	
brown trout	15.5	-	90	Burdick et al. (1958)
Salmo trutta				
fathead minnow	15.0	121	119	Smith et al. (1978)
Pimephales promelas	20.0	137	137	
	25.1	129	129	
bluegill sunfish	8.4	83	61.7	Smith et al. (1978)
Lepomis macrochirus	15.0	87.1	87.1	
	24.9	120	120	
yellow perch	15.0	90.4	87.6	Smith et al. (1978)
Perca flavescens	21.0	102	101	
smallmouth bass	21.0	-	104	Burdick et al. (1958)
Micropterus dolomieui				
largemouth bass	25.0	101	-	Smith et al. (1979)
Micropterus salmoides				
black crappie	25.0	101	-	Smith et al. (1979)
Pomoxis nigromaculatus				

[a] 96-h LC_{50} values of Kovacs (1979), Kovacs and Leduc (1982a), Smith et al. (1978) and Smith et al. (1979) are expressed as HCN; those of Burdick et al. (1958) are of free cyanide expressed as CN.

Table 4-2. Acute toxicity of cyanide to different species of freshwater invertebrates at various temperatures.

Species	Temperature (°C)	96-h LC_{50} ($\mu g.L^{-1}$)	Test Conditions	Reference
snail *Physa heterostropha*	20	432[α]	static	Patrick *et al.* (1968)
isopod *Asellus communis*	18	2,295 HCN	flow-through	Oseid and Smith (1979)
scud *Gammarus pseudolimnaeus*	18	169 HCN	flow-through	Oseid and Smith (1979)
cladoceran *Daphnia magna*	-	400 KCN	static	Towill *et al.* (1978)

[α] Cyanide species not reported.

sensitive to cyanide than are fish, a conclusion also reached by Doudoroff (1980).

Influence of pH

There exists some confusion as to the influence of pH on the toxicity of cyanides to aquatic life. In the range of pH normally encountered in natural waters, pH has no appreciable effect on simple cyanides such as HCN or CN^-. However, small changes of pH will greatly modify the toxicity of metallocyanide complexes.

Considering the dissociation of the weak acid HCN:

$$HCN \rightleftarrows CN^- + H^+ \qquad (4\text{-}9)$$

an increase in pH favors a larger proportion of CN^- (see Section 2.3). It has long been known that HCN is more toxic than the CN^- ion (Wuhrmann and Woker 1948) because of its more facile penetration of the gill epithelium. Only at abnormally high pH can the change in proportion of the chemical species of cyanide be expected to affect its toxicity. Broderius et al. (1977) found that in 96-h LC_{50} tests with fathead minnows, Pimephales promelas, there was no difference in toxicity between free cyanide (HCN and CN^-) and HCN in a pH range of 6.8-8.3. However, above pH 8.3, free cyanide was found to be more toxic than HCN, a fact that was attributed to the CN^- ion penetrating the gills in addition to molecular HCN, thereby enhancing the toxicity of the solution.

Effect of Water Exchange

Because HCN is volatile and readily biodegradable, it presents particular difficulties to bioassay methods. In static tests (i.e. where there is no renewal of the test solution), the concentration of the cyanide solution gradually decreases and tolerance limits tend to be higher than those determined in constant flow tests (i.e. continuous renewal of solution). In a detailed discussion of acute toxicity data, Doudoroff (1976) compared results from "static" and "flow-through" bioassays and noted that, in general, the discrepancies were not very great. With constant flow systems, Doudoroff (1976) found the lethal threshold concentrations and median tolerance limits to fall within the range of 0.05 to 0.16 $mg.L^{-1}$ HCN for cold- and warm-water, nonmarine fishes. In a recent study, Smith et al. (1978) found similar values (i.e. 96-h LC_{50} = 0.057-0.191 $mg.L^{-1}$ HCN) for five species of fish. In the same study, eggs and "swim-up fry" (protopterygiolarval phase, Balon (1975) were more resistant (i.e. 96-h LC_{50} = 0.1-0.35 $mg.L^{-1}$ HCN). Doudoroff (1976) found no evidence that fish are capable of surviving an initial concentration of free cyanide much above 1.0 $mg.L^{-1}$ as CN.

Temperature

The response of aquatic organisms to cyanide at different temperatures has not been thoroughly investigated. It had often been incorrectly assumed that tolerance decreased with increasing temperature. In his review, Doudoroff (1976) examined this assumption with respect to toxicant concentration. When rapidly lethal concentrations were tested, cyanide was generally more toxic at high temperatures (Cairns and Scheier 1963), but at lower concentrations the opposite relationship was observed. This confirmed the earlier work of Wuhrmann and Woker (1955) who found that the effect of temperature on the toxicity of cyanide was dependent on the concentrations used. Recently, Smith et al. (1978), Kovacs (1979) and Kovacs and Leduc (1982a) have clearly shown that cyanide, at relatively low concentrations, is more toxic at a lower temperature.

Smith et al. (1978) tested a wild stock of fathead minnows (Pimephales promelas) at different times of the year at temperatures ranging from 30 to 5°C. A two-way response was observed. From 30 to 15°C, the toxicity decreased, the 96-h LC_{50} changing from 157 to 191 $\mu g.L^{-1}$ HCN. In contrast, from 15 to 5°C the toxicity increased, with the 96-h LC_{50} changing from 191 to 167 $\mu g.L^{-1}$ HCN, respectively.

With brook trout obtained from a single hatchery lot, a positive temperature-tolerance interaction was observed. The 96-h LC_{50} dropped from 143 at 18°C to 53 $\mu g.L^{-1}$ HCN at 4°C. Similarly the lethal threshold concentration (LTC) decreased from 138 $\mu g.L^{-1}$ at 18°C to 52 $\mu g.L^{-1}$ at 4°C. These results are certainly informative, but the lack of data on the thermal history of the two groups of fish is unfortunate. The previous temperature regime of the fathead minnow in the lake is unknown and the duration of acclimatization in the laboratory was not reported. Similar questions are raised concerning the brook trout.

Kovacs (1979) and Kovacs and Leduc (1982a) have also shown that tolerance to cyanide increases with increasing temperature. Juvenile rainbow trout were acclimated for at least two weeks at 6, 12 and 18°C in flow-through test aquaria after which they were exposed to cyanide concentrations in the range of 18 to 87 $\mu g.L^{-1}$ HCN. The results presented in Table 4-3 illustrate that the 96-h LC_{50} increases significantly with increasing temperature. The lethal thresholds could not be measured but were approximated by the determination of the "highest cyanide concentration with no mortality in 96 h". This was related to temperature.

The measurement of survival time also revealed that warm acclimated rainbow trout survived longer in lethal concentrations of cyanide, although the difference in survival time decreased at higher concentrations tested. This trend was further explored by Kovacs (1979) and Kovacs and Leduc (1982a), who drew toxicity curves relating median survival time to a range of concentrations from 20 to 200 $\mu g.L^{-1}$

Table 4-3. Acute toxicity of hydrogen cyanide to rainbow trout acclimated and tested at different temperatures (from Kovacs 1979 and Kovacs and Leduc 1982a).

	Temperature (°C)		
	6	12	18
96-hour LC_{50} and 95% confidence interval ($\mu g.L^{-1}$ HCN)	28 (24-35)	42 (38-46)	68 (64-72)
Slope function	1.30	1.30	1.12
Highest cyanide concentration with no mortality in 96 hours ($\mu g.L^{-1}$ HCN)	18	32	60
Lowest cyanide concentration causing 100% mortality within 96 hours ($\mu g.L^{-1}$ HCN)	37	53	87
Average weight of fish and range (g)	11.79 (9.05-15.98)	12.41 (8.29-16.02)	13.19 (9.63-17.21)
Average fork length of fish and range (mm)	102 (95-110)	103 (90-111)	106 (95-119)
Mean pH and range	8.06 (7.9-8.1)	8.10 (8.06-8.11)	7.82 (7.78-7.90)
Mean oxygen saturation and range (percent)	89.2 (88.2-90.1)	97.1 (95.2-98.5)	86.8 (85.7-88.3)

HCN at 6, 12 and 18°C using their own data as well as those from other researchers. The relationship depicted in Figure 4-2 shows that the acute toxicity of cyanide to rainbow trout at different temperatures is concentration dependent. At low concentrations, the correlation with temperature is positive but becomes negative at rapidly lethal levels. The reversal of the response appears to take place at 100 $\mu g.L^{-1}$ HCN.

At high concentrations of hydrogen cyanide, death probably occurs because of the disruption in the activity of vital organs such as the heart and central nervous system; in this range of concentrations, the toxicant is limited in its speed of action only by the time it takes to get into the blood and circulate to the vital organs (Jones 1964). Time to reach the reaction site (induction time) depends on the rate of uptake of the toxicant and its distribution to the tissues. At higher temperatures, the ventilation volume of fish increases (Heath 1973) together with the metabolic rate, thus accelerating toxicant uptake from the water at the gills and resulting in a faster distribution to the reaction site(s).

At lower concentrations, death from HCN may reflect the disruption of the oxidative processes caused by the inhibition of cytochrome oxidase (see Section 4.2.1) and in this range of concentrations lower temperatures speed up mortality. Only two-thirds of the oxidative processes are sensitive to cyanide (Jones 1964) and a different biochemical makeup at the cellular level and/or metabolic pathways (Hochachka and Somero 1973) in trout acclimated to different temperatures may alter that sensitivity. Ekberg (1958) found that gills of the goldfish (Carassius auratus) showed higher resistance to cyanide poisoning at 30°C than at 10°C, as measured by oxygen consumption. Ekberg (1962) attributed this to a qualitative change of cytochrome oxidase, possibly a stereochemical alteration in the mitochondrial membrane due to temperature. The fact that cytochrome oxidase activity, but not concentration, is greater in cold-acclimated fish may contribute to the lower resistance at lower temperatures (see Section 4.2.1).

At low concentrations, detoxification and excretion could certainly influence the toxicity of cyanide but these mechanisms would also be temperature-sensitive. The detoxification of cyanide due to the enzymatic transformation of CN^- into SCN^- by rhodanese is a well-known mechanism and the protective effect of thiosulfate on cyanide poisoning of carp has long been demonstrated (Achard and Binet 1934). It appears quite possible that the activity of rhodanese and/or the mobilization of sulfur (see Section 4.2.1.5) is enhanced at higher temperatures, thus favoring detoxification and longer survival.

Oxygen

Most organisms will compensate for slightly decreased oxygen levels in the environment by increasing the ventilation volume, and thereby maintaining high O_2 levels internally. If increased ventilation is insufficient, then ATP production by the electron transport chain will decrease, as is the case during cyanide inactivation of cytochrome

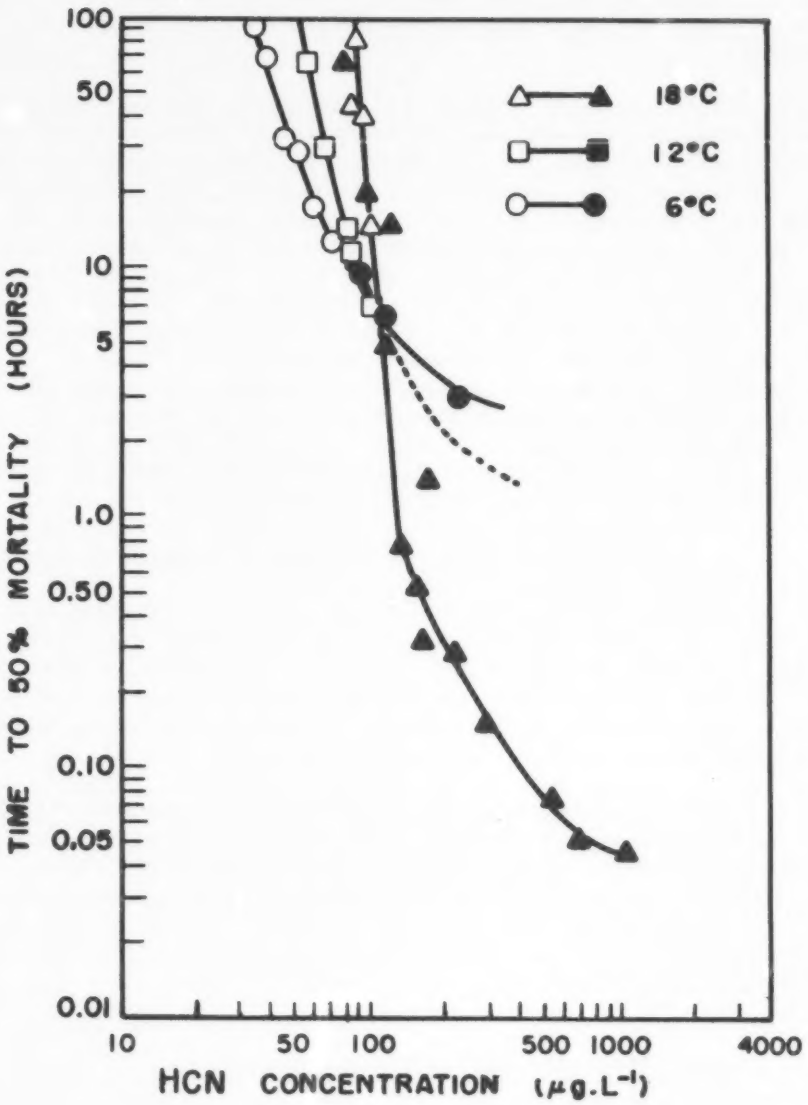


Figure 4-2. Effect of temperature on the toxicity of cyanide to rainbow trout (from Kovacs 1979 and Kovacs and Leduc 1982a).

oxidase (see Section 4.2.1). Therefore, additive effects of oxygen reduction and cyanide intoxication would be expected under certain exposure regimes for organisms incapable of sustained anaerobic metabolism.

The combined effect of decreased oxygen and cyanide is expected to vary with the concentration of cyanide. At extremely high concentrations of cyanide, cytochrome oxidase is inactivated and the effect of oxygen will be minimal. At medium and low concentrations of cyanide, an additive effect upon ATP production may occur in mitochondria, because of reduced environmental oxygen. However, additivity at the site of toxic action may be masked by differences in toxicant uptake at different HCN concentrations. Presumably, increased ventilatory volumes associated with low O_2 in the environment may increase the rate of HCN uptake. Maximum differences in the total volume of water passed over the gills, and hence total cyanide uptake, may be manifested at low cyanide concentrations by a synergistic (i.e. greater-than-additive) oxygen-cyanide interaction. In this case, synergism pertains to ambient concentrations; the cyanide dose concomitant with reduced oxygen concentrations in the tissues may interact according to toxicant additivity.

Perhaps the most striking example of synergism was observed by Downing (1954). At the lowest concentration of cyanide used (0.105 $mg.L^{-1}$ as CN) there was a hundredfold decrease in the median survival time with a decrease in dissolved oxygen from about 9 to about 3 $mg.L^{-1}$. However, at higher levels of cyanide, causing fish to become immobilized within minutes, the effect of ambient oxygen concentrations was only slight. Wuhrmann and Woker (1955) and Burdick et al. (1958) observed similar results.

More recently, several freshwater fish species were tested and lethal thresholds were reduced 20-30% when the oxygen concentration was reduced by 50% (Smith et al. 1978). In the same study, brook trout alevins, although much more resistant than adults, showed a much higher sensitivity when oxygen was reduced. Alevins having yolk sacs exhibited a 500% decrease in lethal threshold concentration while those which had resorbed their yolk sacs exhibited a 200% decrease.

Ionic Strength: Salinity and Hardness

In general, hardness of the water does not appear to have any significant effect on the toxicity of HCN. There is little information on this subject and most of it is contradictory. Doudoroff (1976) suggested that apparent hardness effects may be interpreted to mean that alkalinity has indirectly influenced toxicity through the control of pH (i.e. carbonate-bicarbonate buffering system in water).

Salinity may produce a significant reduction in tolerance to HCN only when fresh water and full strength seawater are compared;

dilute seawater and 50% seawater may have little effect upon survival (Doudoroff 1976). Although information is limited, the energetic cost of osmotic and ionic regulation in seawater may be greater than that in fresh water. Sensitivity to cyanide may therefore be greater in seawater (see Section 4.2.3.6).

Broderius (1973) observed that the toxicity of NaCN to the threespine stickleback, Gasterosteus aculeatus, increased with salinity. The median survival time at a nominal concentration of 270 $\mu g.L^{-1}$ as CN and full strength seawater was half that of fish tested in fresh water. The physiological significance of these observations remains obscure, however, since no physical parameters other than death were measured.

Acclimation

The wide occurrence of cyanide/cyanogenic wastes may create situations in which organisms are continuously exposed to subthreshold levels, or periodically exposed to sublethal levels. Do organisms alter their resistance to cyanide in such situations? The few studies reviewed by Doudoroff (1976) indicated that low sublethal levels can increase resistance to a lethal concentration somewhat, but that higher exposure concentrations may result in decreased resistance.

Recently, Dixon (1980) and Dixon and Sprague (1981) established that high sublethal levels initially decrease resistance, the effect diminishing with time. Rainbow trout were preexposed to 34 $\mu g.L^{-1}$ HCN for 7, 14, and 21 days and the resistance to acute poisoning was compared with that of fish that had not been preexposed. The results (Figure 4-3) demonstrate a substantial drop in resistance after 7 days, followed by a gradual return to control values after 21 days. Associated biochemical changes are unknown, but it is interesting to note that the time frame is equivalent to that for changes in resistance at the sublethal level of exposure (see Section 4.2.3). Specifically, growth of cyanide-exposed fish was initially depressed, but rebounded after 10 days.

Body Size

As an antimetabolite cyanide has been shown to follow the same weight related response as was observed in metabolic studies by Anderson and Weber (1975). The metabolic rate of fish is related to its size (Winberg 1956) and depending on the species and age, this relationship may or may not be proportional. Nevertheless, these relationships between metabolic rate and size can collectively be described by the formula:

$$y = ax^b \text{ (Bertalanffy 1957)} \qquad (4\text{-}10)$$

where y = oxygen consumption/fish/unit time

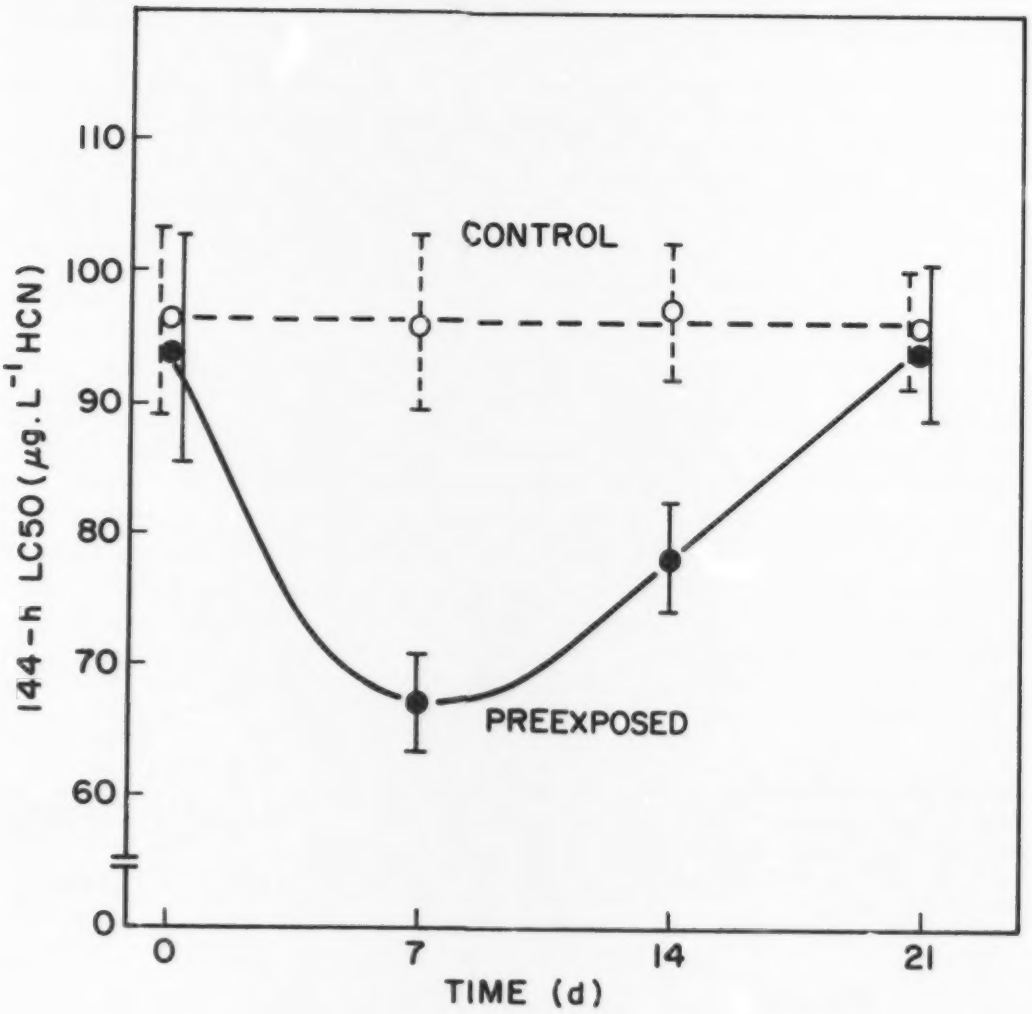


Figure 4-3. Effect of preexposure to 34 μg.L^{-1} HCN over a 21-d period on the 144-h LC_{50} of rainbow trout (95% fiducial limits are given) (from Dixon 1980 and Dixon and Sprague 1981).

x = size of the fish, usually measured as weight

b = exponential function of x that describes the proportionality or nonproportionality

a = constant determined by the environmental conditions governing the tests

Equation (4-10) may be transformed to:

$$\log y = \log a + b \log x \qquad (4\text{-}11)$$

which allows the exponent to be defined by the slope of a straight line fitted to log-distributed data relating metabolic rate to size. Winberg (1956), using his own laboratory data and others, suggested that the metabolic rate of all groups of fish could be related to size by an exponential factor approximating 0.7-0.8.

Anderson and Weber (1975) have tested the relationship of mortality-concentration with potassium cyanide from 96-h LC_{50} bioassays carried out on guppies (Poecilia reticulata) of various size groups. The 96-h median lethal cyanide concentration in $mg.L^{-1}$ CN for fish of a given weight class was shown to be $0.147 \cdot W^{0.72}$ where W is the weight in grams of a group of 10 fish of a given size class. From this equation it follows that for a 1 g biomass the LC_{50} is 0.147 $mg.L^{-1}$ whereas for a 10 g biomass of larger fish the LC_{50} would be 1.297. The biological significance of these experiments is that the LC_{50} is weight-related and should be expressed per weight (i.e. a weight specific LC_{50}) just as oxygen consumption is expressed per gram of fish and not for the total respiring biomass. In the above example, the $LC_{50}.g^{-1}$ in the first case is 0.147 $mg.L^{-1}$ CN, but in the second, 0.1297. As Anderson and Weber (1975) said: "In common parlance this means that...less toxicant was required to kill 1 g of heavier fish than for 1 g of lighter fish."

The allometric response of fish to respiratory deficiency has been established. Wells (1913) found that the dying time per gram of the common shiner (Notropis cornutus) in oxygen deficient water decreased from 25.0 to 2.1 minutes with an increase in size from 0.6 to 21.0 g. Herbert and Merkens (1952) observed that the survival time of juvenile rainbow trout subjected to a lethal solution of cyanide decreased from 39.0 to 16.0 minutes with an increase in length from 5.75 to 17.25 cm. Hence, the lethal studies concerning low levels of oxygen and the inhibition of utilization of oxygen by cyanide both show that smaller fish are more tolerant than larger ones.

The value of 0.7 for b in equations (4-10) and (4-11) suggests a surface area to weight function, and some authors have concluded that metabolic rate for different size fishes is a function of the surface area of the gills at which O_2 is exchanged. Exploring this possibility,

Hughes (1970) found that for several groups of fish, the change in gill area with increase in size could be described by an exponential factor of 0.8 in equation (4-10), showing that smaller fish have a larger gill area per unit weight than larger ones. In smaller fish, the greater oxygen consumption per unit weight (Beamish 1964) and lower cost of gill ventilation (Jones 1971) may help to explain how they are better able to cope with cyanide exposure.

4.2.3 CHRONIC EXPOSURES

4.2.3.1 Reproduction

Despite the crucial importance of recruitment to the stability of fish populations, there is only limited knowledge pertaining to the effects of cyanide on reproduction. Several studies in which exposure has continued throughout the life cycle have demonstrated that decreased egg production is a more sensitive indication of sublethal effects than growth inhibition or survival of larvae. However, teratogenic effects are known to occur at low levels of cyanide and may be elicited below the threshold for reproductive impairment.

The intricate behavioral patterns associated with successful reproduction are apparently unaffected by cyanide at levels known to cause reproductive impairment (Brockway 1963). Cyanide may, therefore, operate upon physiological target sites in adults and embryos. More detailed studies have investigated the possibility that gametogenesis in adult fish is the primary target.

In a life-cycle study, Lind *et al.* (1977) exposed fathead minnows (*Pimephales promelas*) of both sexes for 265 days through the F_1 generation at 25°C, testing cyanide concentrations from 5.7 to 105 $\mu g.L^{-1}$ HCN. The number of eggs produced per female was reduced by 50% at approximately 15 $\mu g.L^{-1}$ HCN (Figure 4-4). The effects of cyanide on the reproduction of a cold-water species, brook trout (*Salvelinus fontinalis*), was investigated by Koenst *et al.* (1977). Following a 144-day exposure at 9-15°C, reproduction was observed to have been seriously affected (Figure 4-4). Egg production per female was reduced by 50% at approximately 27 $\mu g.L^{-1}$ HCN.

Kimball *et al.* (1978) concluded that, for bluegill sunfish (*Lepomis macrochirus*), spawning is completely inhibited at 5.2 $\mu g.L^{-1}$ HCN. However, this investigation requires corroboration because no dose response relationship was delineated and because fecundity (total number of eggs produced by a female) of the control fish was approximately 20% of that demonstrated by control bluegills in other toxicological investigations (cf. Benoit 1975).

There is no indication in the results of these studies as to how the male and female gonads may have been affected. A meaningful

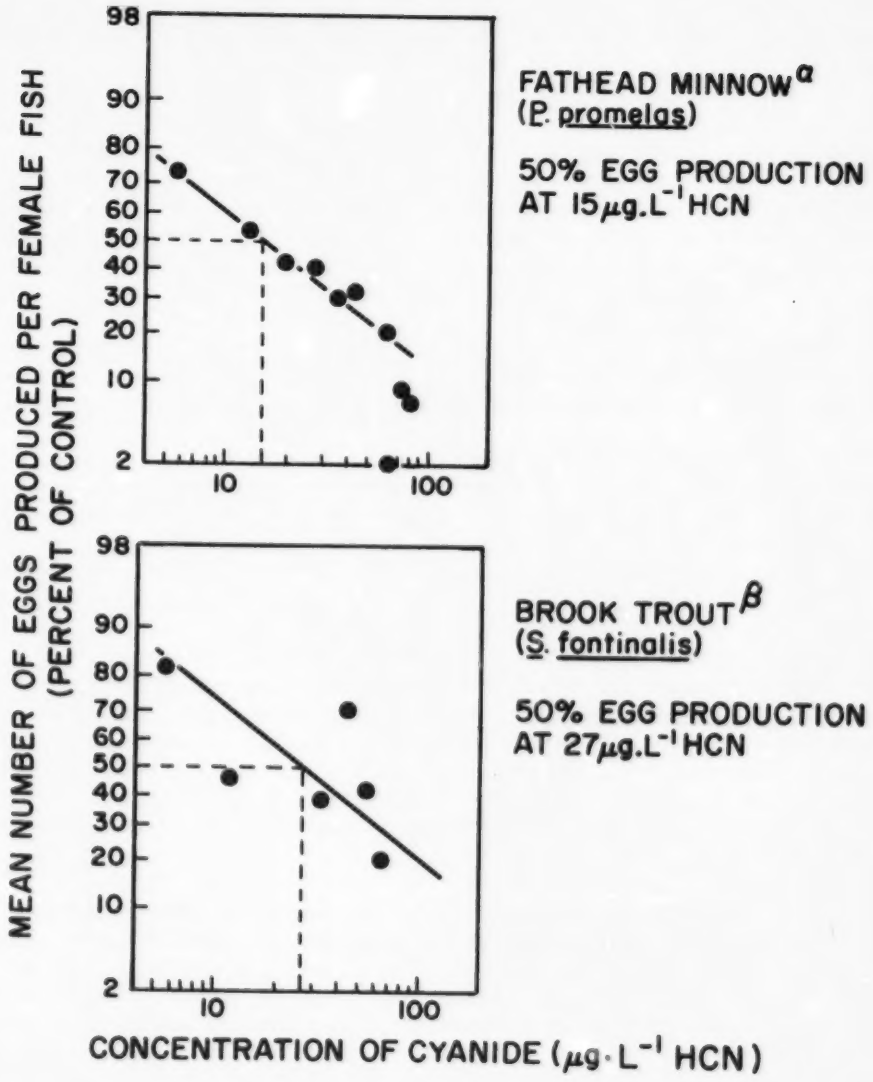


Figure 4-4. Effect of HCN upon reproduction of two species of freshwater fish, P. promelas and S. fontinalis (see text for discussion).

α From Lind et al. (1977)

β From Koenst et al. (1977)

approach to this question lies in the detailed histological study of these organs. Along these lines, Lesniak and Ruby (1982) demonstrated that cyanide, at 10 $\mu g.L^{-1}$ HCN, could seriously reduce the maturation of oocytes in yearling rainbow trout (Salmo gairdneri) exposed to HCN for periods as short as 15 to 20 days; the yolk deposition stage was most seriously affected.

Histological examination of the ovaries at the light microscope level revealed several oocyte abnormalities (Lesniak and Ruby 1982). These included delayed Balbiani dispersion and increased PAS-positive granules, suggesting an interruption in utilization of protein and muco-polysaccharides within the cytoplasm and a subsequent effect upon yolk deposition. In both experiments, the females exposed to 10 $\mu g.L^{-1}$ HCN displayed a unique form of atresia which was present in all stages of oocyte development. At the ultrastructural level, cyanide-exposed oocytes revealed, within the mitochondria, characteristics of both early and advanced pathological conditions, suggesting an interruption in the energy-producing mechanisms due to cyanide.

By the establishment of six arbitrary stages of oocyte development (Table 4-4) the effects of cyanide were quantitatively assessed, as illustrated in Figure 4-5. This histogram reveals a significant increase in stage 3 oocytes and indicates that fewer eggs than in controls developed beyond the Balbiani protein deposition. That was further substantiated by a 35%-38% lower frequency of secondary yolk stage 5 oocytes compared with that of the controls. Oocytes that fail to complete development become atretic and are resorbed. The frequency of these oocytes (stage A) was markedly increased in the presence of cyanide. Furthermore, the growth of oocytes was monitored during the exposure period and based upon the maximum diameter of oocytes in a given ovary section, the data were interpreted to indicate that the egg maturation rate was retarded in cyanide-exposed fish. Such effects might manifest themselves through delayed spawning or decreased numbers of viable eggs.

Further to these studies on female rainbow trout, Ruby et al. (1979) reported the effects of cyanide on male gonads. This study involved a histological approach to assess, quantitatively and qualitatively, the impact of an 18-day exposure of juvenile male trout (Salmo gairdneri) to cyanide in laboratory flow-through aquaria at 12.5°C. The two test concentrations, 10 and 30 $\mu g.L^{-1}$ HCN, reduced the number of dividing spermatogonia by 13% and 50%, respectively (Figure 4-6). Not only was mitotic activity hampered by cyanide, but none of the cells that entered mitosis reached the anaphase stage. Instead, they accumulated in the prophase stage, where markedly higher numbers were counted in the testes of cyanide-exposed fish than in those of the controls.

Cyanide was also observed to affect the formation of the mitotic spindle at the lower cyanide concentration as evidenced by the presence of multipolar spindles and multinucleate interphase cells.

Table 4-4. Summary of morphological criteria defining arbitrary stages of oocyte development in the ovaries of rainbow trout (from Lesniak and Ruby 1982).

Stage	Oocyte diameter (μm)	Nucleus	Nucleoli Mean Number/8-μm Section	Cytoplasm	PAS[α]	Membranes	Follicular Layer
0	4-6.5	round, strongly basophilic	none visible	rim, pale basophilic	-	indistinct	none apparent
1	6.5-20	round, large, pale	none distinct from chromatin	opaque, lightly basophilic	-	distinct nuclear, indistinct cytoplasmic	incomplete spindle-shaped follicular cells
2	20-80	ellipsoidal, eccentric	9 large, few scattered	strongly basophilic Balbiani, idosome and pallial layers	-	nuclear and cytoplasmic, developing zona pellucida	incomplete squamous layer of follicular cells
3	80-160	ellipsoidal, similar	21 mixed sizes, migrate to periphery	dispersing and fragmentation of Balbiani body, primary yolk vesicles	+	completed zona pellucida	thin, complete simple follicular layer
4	160-240	ellipsoidal, central, indented	35 small, peripheral	secondary yolk vacuoles form a band at the nuclear membrane and another in the peripheral cytoplasm	++	thickened zona pellucida, nucleus indented	inner layer epithelial cells, outer thecal cells, both single squamous
5	240	irregular, moves to one pole	up to 53, small, follow nuclear outline	coalesced yolk globules, acidophilic	++	wavy, thick zona pellucida, broken nuclear	follicular layer of cuboidal cells

[α] (-) negative and (+) positive reaction to periodic acid-Schiff stain. Total sample size was 20 eggs per stage.

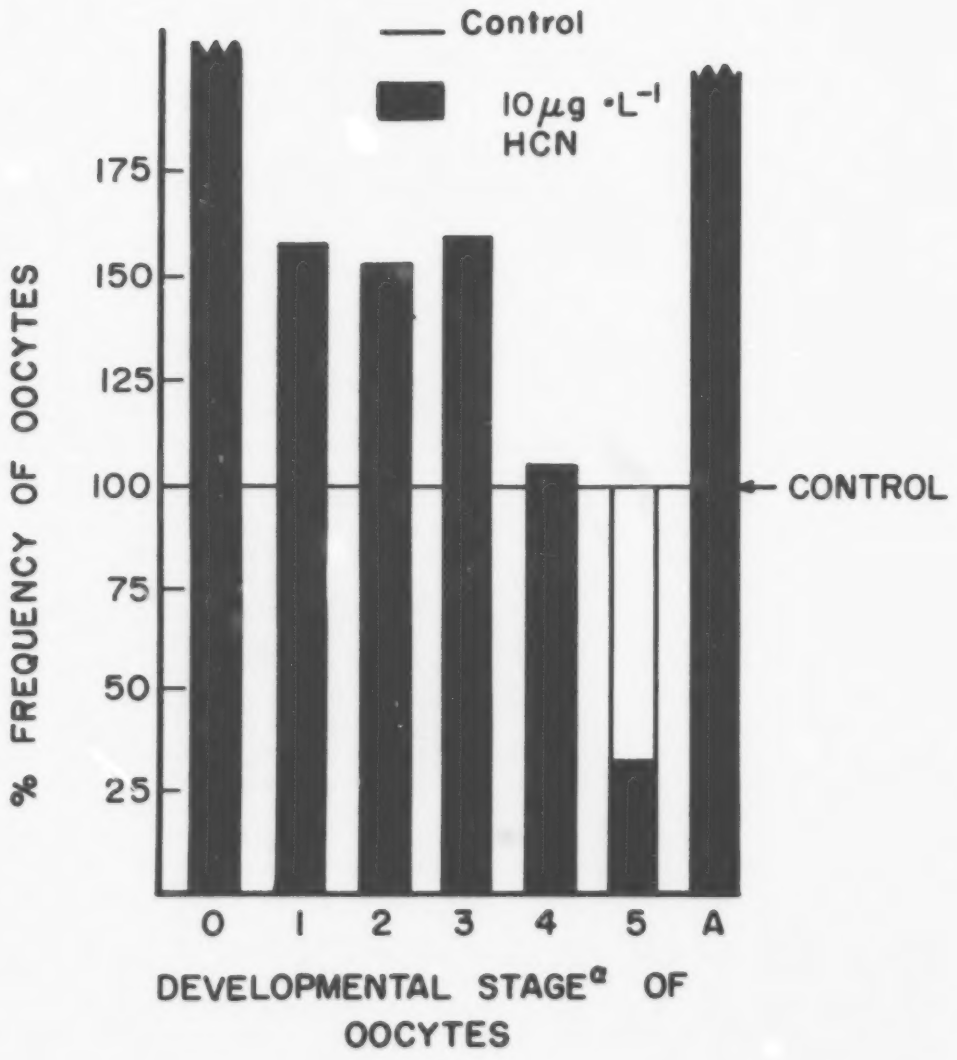


Figure 4-5. Effect of cyanide on oogenesis in rainbow trout exposed for 15-20 days at a concentration of 10 $\mu g.L^{-1}$ HCN. The % frequency of oocytes of the various developmental stages is measured relative to a control value of 100% (from Lesniak and Ruby 1982).

[α] Developmental stages as defined in Table 4-2. "A" represents atretic oocytes.

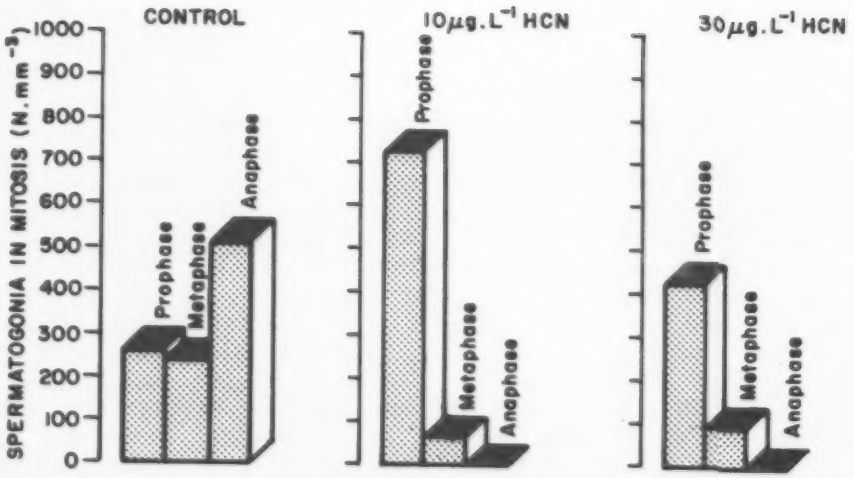


Figure 4-6. Effect of cyanide on spermatogenesis in rainbow trout exposed for 18 days to concentrations of 10 and 30 $\mu g.L^{-1}$ HCN (from Ruby et al. 1979).

Cellular damage, evident in spermatogonia in all phases of the cell cycle, was accompanied by a high incidence of necrobiosis at the higher cyanide concentrations. It is proposed that short-term exposure to low concentrations of cyanide could cause permanent damage to the fixed number of primary spermatogonia within the testes, thereby reducing the reproductive capacity of this species.

4.2.3.2 Development During Early Life Phases

Eggs of the mummichog, Fundulus heteroclitus, underwent normal cleavage, even at cyanide concentrations up to 520 $mg.L^{-1}$ CN, until blastulation was complete (Wilde and Crawford 1963; Crawford and Wilde 1966a). Thereafter, morphogenesis and cell differentiation became entirely aerobic, hence increasing the embryo's sensitivity to cyanide. Higher lactic acid levels and reduced amounts of ATP were observed in test embryos compared with those of the controls (Crawford and Wilde 1966a). In addition, RNA synthesis was completely arrested (Crawford and Wilde 1966b).

There are a few other reports (cf. Doudoroff 1976) of the effects of high concentrations of cyanide on fish embryos but they are almost entirely restricted to observations of early cleavage, oxygen consumption and heart beat. In contrast to these, a study was conducted in which two generations of fathead minnows (Pimephales promelas) were exposed continuously to various concentrations of cyanide (Lind et al. 1977). In the control tanks of the first generation, mortality among larval minnows averaged 36% (range: 29-42%) in the first 28 days of the experiment. This compares with 71% to 81% mortality at cyanide concentrations greater than 67 $\mu g.L^{-1}$ HCN (i.e. 76-100 $\mu g.L^{-1}$ HCN). When the surviving fish grew to maturity and spawned, hatchability was significantly reduced at 44 $\mu g.L^{-1}$ HCN. In the control tanks of the second (F_1) generation, larval mortality averaged 16% (range: 15-17%) in the first 28 days. At 81 $\mu g.L^{-1}$ HCN, larval mortality had reached 64% within the same time interval. Larval growth was inhibited at 58 $\mu g.L^{-1}$ HCN in the first generation compared with 35 $\mu g.L^{-1}$ HCN in the second generation. Growth effects at these concentrations were apparently sustained through the larval phase while transient growth effects were evident at lower cyanide levels.

The results of Lind et al. (1977) would suggest that the fish had become slightly more sensitive to cyanide in the second generation. However, the results are difficult to interpret because some of the eggs and/or larvae used in the second generation exposure originated from nonexposed parents. Also, it is not possible to tell whether or not the hatching success was due to the treatment prior to, or after spawning, or to both.

Although brook trout (Salvelinus fontinalis) were found to spawn successfully following chronic exposure to 65 and 75 $\mu g.L^{-1}$ HCN

(see Section 4.2.3.1), none of the eggs produced at these concentrations developed to the neural keel stage (Koenst et al. 1977). Lower cyanide concentrations may have caused embryo mortality prior to hatching, but the results pertaining to hatchability are inconclusive.

The effects of cyanide on Atlantic salmon (Salmo salar) eggs were studied by incubating fertilized eggs at 2.7°C and 4.4°C in concentrations ranging from 10 to 100 $\mu g.L^{-1}$ HCN (Leduc 1978). All cyanide concentrations retarded hatching, but appreciable delays (6-9 days) occurred only at 80 and 100 $\mu g.L^{-1}$ HCN. There was a definite trend toward reduced hatching success with increasing concentrations, and continuous exposure of the eggs caused hatching failure of 15% to 40%. Of the individuals that hatched successfully, numerous abnormal eleutheroembryos ("yolk-sac fry") were observed. Abnormal eleutheroembryos were virtually absent from the controls but ranged from 5.8% to 18.5% for HCN-exposed fish. The anomalies included malformations such as absence of eyes, defects in the mouth and the vertebral column, and yolk sac dropsy (Hydrocoele embryonalis). After hatching, the survival of eleutheroembryo salmon was not affected by cyanide. However, the growth of eleutheroembryo and alevin salmon was temporarily inhibited by 40, 80 and 100 $\mu g.L^{-1}$ HCN. By 58 days post-hatching, growth at 100 $\mu g.L^{-1}$ HCN had increased and approached that of controls. Growth at lower cyanide levels had accelerated beyond that of controls. Weight gains essentially paralleled the changes in body length.

In the same study, the efficiency of yolk conversion into body tissue was estimated by measuring the initial yolk content and the dry weights of eleutheroembryos (with and without yolk sacs). During incubation, yolk conversion efficiency was reduced at HCN concentrations >20 $\mu g.L^{-1}$. After hatching, this trend was reversed. Yolk conversion efficiency apparently increased with increasing concentrations of cyanide; markedly higher efficiencies were observed for HCN concentrations >40 $\mu g.L^{-1}$.

The fact that cyanide-exposed salmon demonstrated a greater yolk-conversion efficiency while outgrowing the controls may be explained by their relative inactivity. Individuals affected by 80 and 100 $\mu g.L^{-1}$ HCN rested on the bottom of the tanks and remained in stationary positions despite disturbances imposed by the researcher. Thus, the exposed fish would have been highly susceptible to predation. In contrast, controls were generally more mobile and did not hesitate to swim actively when disturbed. The increase in yolk conversion efficiency on the part of inactive eleutheroembryos may therefore indicate physiological stress or "poor viability" (Blaxter and Hempel 1966).

The total impact of cyanide on the early life phases of Atlantic salmon is summarized in Figure 4-7. The index, "Realized Viability", was calculated as the number of deaths plus the number of

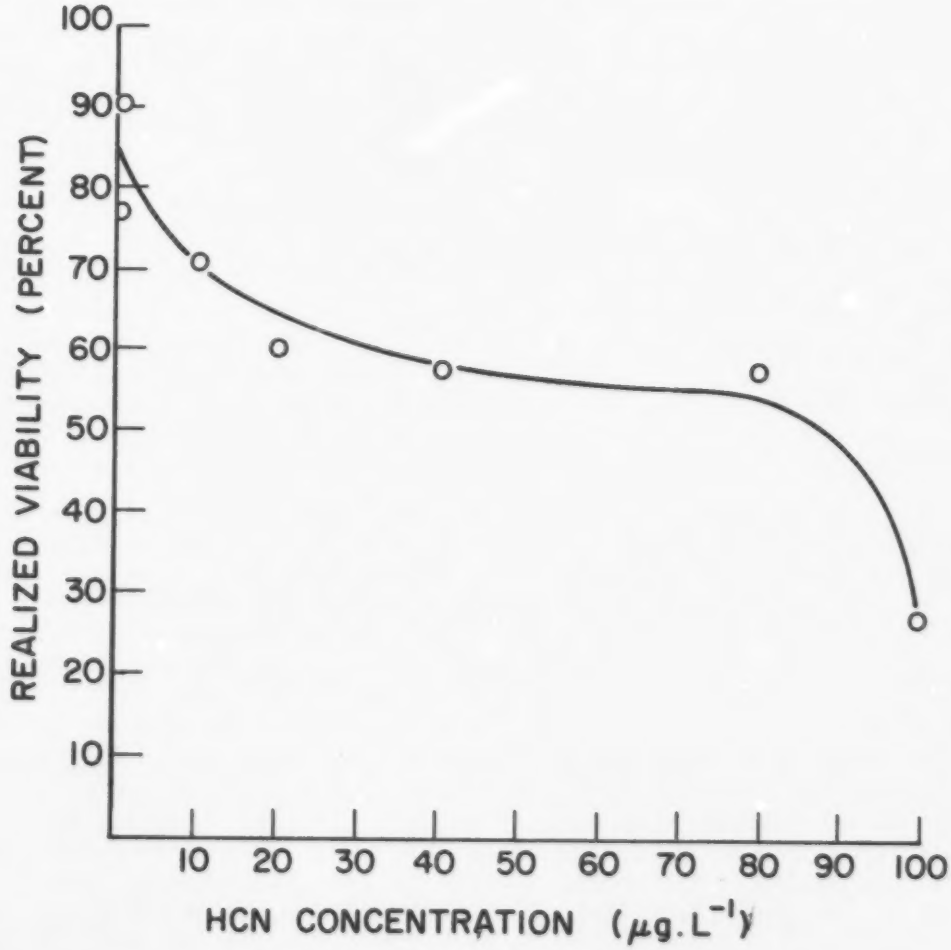


Figure 4-7. Realized viability of Atlantic salmon at different HCN concentrations to which eggs and eleutheroembryos were constantly exposed (from Leduc 1978).

abnormalities as a percentage of the number of individuals exposed to a given cyanide concentration. Figure 4-7 shows that 10 $\mu g.L^{-1}$ HCN is above the threshold for detrimental effects to salmon embryos and eleutheroembryos. At 90 $\mu g.L^{-1}$ HCN the realized viability was reduced to 50% of the control value.

Various effects of cyanide upon developing flagfish (Jordanella floridae) have been documented by Cheng (1978) and Cheng and Ruby (1981). Control eggs incubated at 25°C were found to hatch after 114 hours. Eggs exposed to 65 $\mu g.L^{-1}$ HCN hatched 26 hours later, and a delay of approximately 30 hours occurred at concentrations ranging from 75 to 87 $\mu g.L^{-1}$ HCN. Hatching success was reduced by 20-40% in the range 75 to 87 $\mu g.L^{-1}$ HCN and a high incidence of teratogenic effects was observed at 65, 75 and 87 $\mu g.L^{-1}$ HCN. The anomalies, detected in 14-day old larvae, included various eye defects (30-40% incidence) and a decrease in size of the pituitary gland.

The hatching success and survival of the F_1 fry were comparable to that of controls. However, the early exposure to cyanide during embryogenesis had accelerated growth of the juvenile fish but retarded their sexual development (Cheng 1978). The test fish began to spawn 70 days after hatching, 14 days later than the controls. In addition, the cyanide-exposed fish suffered a shortened estrous cycle and laid a significantly smaller number of eggs than did the controls.

4.2.3.3 Growth

As a respiratory poison, cyanide would be expected to reduce the energy available for growth (Warren et al. 1973), as seems to be the case for many exposure conditions. The extent to which energy may be diverted from growth has been calculated from the data of McCracken (1978) and McCracken and Leduc (1980) and is expressed as the rates of energy transfer from the food and body reserves of fish (Figure 4-8). The situation represented is one of growth restriction by food (i.e. 1.5% food ration) and by imposed environmental conditions (i.e. constant swimming at 1 body length per second). The energy required for metabolism in the absence of HCN is 32.5 $cal.g^{-1}.d^{-1}$, whereas the requirement more than triples (i.e. 115.8 $cal.g^{-1}.d^{-1}$) under chronic exposure to 10 $\mu g.L^{-1}$ HCN. The greater energy demand is most likely associated with the blockage of a proportion of ATP produced via electron transport and the partial reliance upon glycolysis. The energy available from the food is considerably less than the metabolic requirement, meaning that the exposed fish would eventually succumb to starvation. (Further details concerning the energy budget are found in Appendix I. It should be noted that because several assumptions were made in deriving the energy budget, that it is only a general approximation).

In apparent contrast to the action of HCN upon respiration, growth increases have been repeatedly observed. One may speculate that in such cases a thyroid response may have been elicited (see also

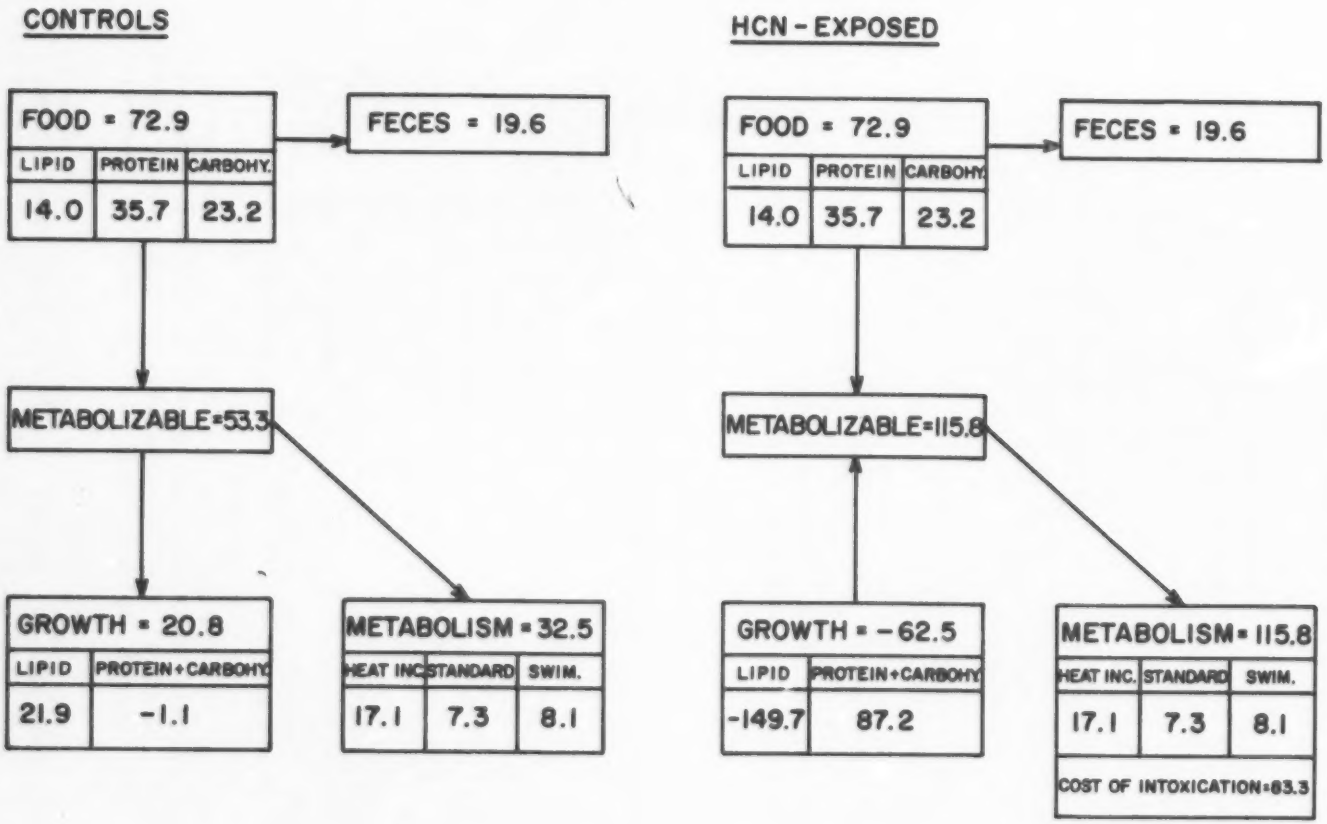


Figure 4-8. Effect of HCN upon rates of energy transfer[α] from food and body reserves of fish (data from McCracken 1978 and McCracken and Leduc 1980).

[α] Values of energy transfer expressed as $cal.g^{-1}.d^{-1}$.

HEAT INC. = heat increment
SWIM. = metabolic requirement for swimming
CARBOHY. = carbohydrate

Section 4.1). The pattern of response to low levels of cyanide is typical of that associated with thyroid hormones in mammals (Tata 1974). Specifically, slight hyperthyroidism causes enhancement of anabolic responses, whereas hypothyroidism and pronounced hyperthyroidism are inhibitory or catabolic. A relationship between cyanide or thiocyanate exposure and thyroid effects has yet to be demonstrated experimentally for fish.

The study of the effects of cyanide on growth was initiated by Leduc (1966) working with cichlid fish (Cichlasoma bimaculatum) fed unlimited rations of live tubificid worms. The cichlids were held in rectangular troughs, supplied with spring water heated to 25°C, and subjected to various cyanide concentrations ranging from 10 to 100 $\mu g.L^{-1}$ HCN for 36 days. Growth was measured as changes in wet weight.

Cyanide had different effects on growing cichlids. The effects varied with time of exposure and with the concentrations tested. Cyanide promoted a higher food consumption; food conversion efficiency was initially higher at cyanide levels around 20 $\mu g.L^{-1}$ but lower at concentrations > 20 $\mu g.L^{-1}$ HCN. The resulting growth was then initially enhanced at low cyanide levels but inhibited at higher cyanide levels (Figure 4-9). With time this pattern changed such that fish at the lesser cyanide concentrations lost their growth advantage, whereas those at the greater concentrations grew better. By the end of the 36-day period there were hardly any differences between the control and the cyanide-exposed cichlids except for a slight depression at 100 $\mu g.L^{-1}$ HCN (Figure 4-9). Similar results were obtained by Leduc (1966) with coho salmon (Oncorhynchus kisutch) fed an unlimited ration of earthworms in a flow-through system at 16°C.

Dixon (1975) and Dixon and Leduc (1981) exposed young rainbow trout to 10, 20 and 30 $\mu g.L^{-1}$ for two successive periods of 9 days in each of 2 experiments at 12°C while feeding them daily rations of 2.0% and 2.5% of wet body weight. Cyanide had an initial drastic effect, causing an almost complete arrest of growth at 30 $\mu g.L^{-1}$, but again, the growth of cyanide-exposed trout showed a marked rebound in the second 9-day period. However, this response was not sufficient to compensate for the initial depressive effect of cyanide. Exposure to cyanide for 18 days resulted in significant reductions of growth at 20 and 30 $\mu g.L^{-1}$ HCN. Speyer (1975) reached similar conclusions with rainbow trout tested at 20 $\mu g.L^{-1}$ HCN and 11°C. Using flagfish (Jordanella floridae), Cheng (1978) demonstrated growth enhancement in juveniles that had been exposed to cyanide during embryogenesis (Section 4.2.3.2). Hence it appears that there are some specific species differences in sensitivity to cyanide (Figure 4-9).

However, temperature could also have played a major role since cichlids and flagfish were tested at 25°C, coho salmon at 16°C and rainbow trout at 11°C. Kovacs (1979) and Kovacs and Leduc (1982b) have recently investigated this possibility while carrying out a series of

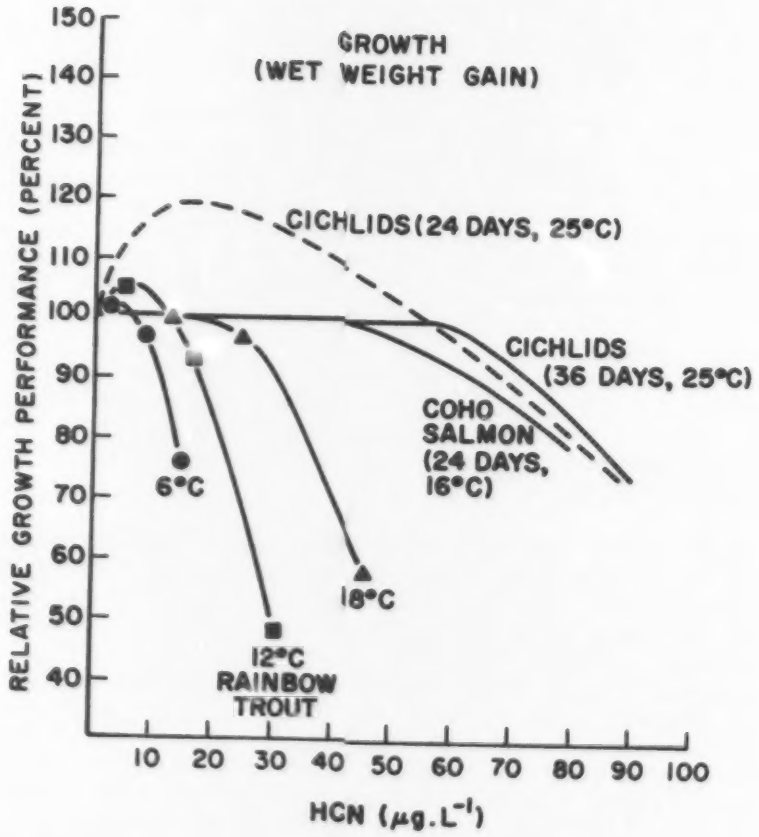


Figure 4-9. Relative growth index of various species of fish chronically exposed to HCN. The studies with cichlids and coho salmon were performed by Leduc (1966); rainbow trout were tested by Kovacs (1979) and Kovacs and Leduc (1982b).

20-day growth experiments on juvenile rainbow trout at various sublethal concentrations of cyanide ranging from 5 to 45 $\mu g.L^{-1}$ HCN at 6, 12 and 18°C. In all experiments the fish had been acclimated to the test temperature in flow-through tanks for at least three weeks prior to the beginning of the tests.

Kovacs (1979) and Kovacs and Leduc (1982b) clearly showed that the effects of cyanide on growth were greater at lower temperatures (Figure 4-9). At 6°C, fish exposed to 15 $\mu g.L^{-1}$ HCN for 10 days had wet body weights that were 51% less than those of the controls. The relative growth of HCN-exposed individuals was slightly greater in the subsequent 10-day period, resulting in wet weights 22% less than those of control fish. At 12°C, trout exposed to 20 and 30 $\mu g.L^{-1}$ HCN grew 29 and 75% less than controls on a wet weight basis during the first 10 days. Growth was slightly greater during the 10- to 20-day period. At 18°C, the wet weights of trout exposed to 45 $\mu g.L^{-1}$ HCN averaged 50% less than the weights of controls for the first 10-day period. This trend did not change substantially during the next 10 days, resulting in an overall difference of 41%. Wet weights were apparently unaffected by lower cyanide concentrations at 18°C.

Fish growth may be measured by changes in dry body weight since it is often a better indication of net tissue deposition. The measurement of dry weights at different temperatures and cyanide concentrations revealed a marked effect of both factors on water uptake. Water contents were higher in control fish at lower temperatures and this phenomenon was enhanced by cyanide. When dry-weight gains were used to assess growth, it was found that concentrations as low as 10 $\mu g.L^{-1}$ could reduce dry weight gain by 18%. At 12°C, 30 $\mu g.L^{-1}$ reduced dry weight gain by 70% whereas at 18°C the same concentration lowered it by about 40%.

The marked effect of cyanide on dry weight gain was due presumably to its impact on the fat content, which was drastically reduced. Reductions of fat content were 40-75% at 6°C, 20-80% at 12°C and 12-50% at 18°C.

The overall effect of cyanide at different temperatures is summarized in Table 4-5 where threshold concentrations at different temperatures are given separately for wet weight, dry weight and fat weight gains. It can be seen that the threshold values increased with temperature by a factor of 3 in the range of 6 to 18°C for wet weights and by a factor of > 5 for dry and fat weights. The change in toxicity of cyanide follows the Q_{10} law of metabolic activity at different temperatures (Giese 1973). For wet weight gains, the change in threshold values from 6 to 18°C, corresponds to a Q_{10} of 2.5.

The causes of the higher chronic toxicity of cyanide at low temperatures have not been thoroughly investigated but 3 points merit consideration:

Table 4-5. Effect of HCN exposure at different temperatures upon wet weight, dry weight and fat weight of rainbow trout (from Kovacs 1979 and Kovacs and Leduc 1982b).

	Threshold concentration ($\mu g.L^{-1}$ HCN)		
growth measurement	6°C	12°C	18°C
wet weight	10	15	30
dry weight	<5	10	30
fat weight	<5	<10	25

1) At low temperatures, cyanide may affect the conversion of food into energy required to perform routine metabolism. Kovacs (1979) found that rainbow trout exposed to 15 $\mu g.L^{-1}$ HCN at 6°C responded by a rapid utilization of glycogen and the production of lactic acid as a source of anaerobic energy. This response was delayed at 12°C and nonexistent at 18°C.

2) Low temperature normally increases the water content in freshwater fish because of reduced renal excretion, and cyanide produces a similar effect (Dixon 1975; Speyer 1975; Kovacs 1979). By interfering with the water balance in fish, cyanide may impose an additional stress to the osmotic stress associated with low temperature.

3) Cytochrome oxidase activity is found to be greater in fish acclimated to cold water (Section 4.2.1).

McCracken (1978) and McCracken and Leduc (1980) investigated some of the interactions between growth effects due to cyanide and the activity, food ration, and size of the fish. Using a series of annular growth chambers equipped with motor-driven paddle wheels to maintain a constant current (Kruzynski 1972), the effects of cyanide at 10 $\mu g.L^{-1}$ were measured, using different food rations, on juvenile rainbow trout swimming at 12 $cm.s^{-1}$ at 10°C.

The rate of growth of fish, initially weighing 18 g, was significantly reduced by cyanide exposure at all food rations tested (i.e. 0.5, 1.0 and 1.5% of wet body weight per day) and the food maintenance requirement (the amount of food required for a fish to neither gain nor lose weight) was markedly increased. In subsequent experiments, McCracken (1978) and McCracken and Leduc (1980) demonstrated that the food maintenance requirement was not affected in smaller trout (8 g) exposed to cyanide. For the large fish, cyanide increased the food maintenance requirement from 0.45% to 0.65% per day based on wet weight gain and from 0.5% to 1.5% per day based on dry weight gain. This apparent size-related response to chronic cyanide poisoning was investigated by systematically testing the growth of rainbow trout ranging in size between 5 g and 30 g. The growth of smaller fish was stimulated by cyanide over that of the control group, whereas the growth of larger fish was depressed (Figure 4-10). For fish of 10 to 15 g there was no difference. The size-dependency of the growth response to cyanide was interpreted by McCracken (1978) and McCracken and Leduc (1980) to mean that it was influenced by the different metabolic rates between small and large fish.

Alternatively, the differential effect of cyanide on large and small fish was explained on the basis of size-related differences in fat gain (McCracken 1978; McCracken and Leduc 1980). Figure 4-11 shows gain or loss of fat in the control and cyanide-exposed fish used in the regression analysis (Figure 4-10). From the curve representing the controls, it can be seen that the small fish increasingly gained fat up

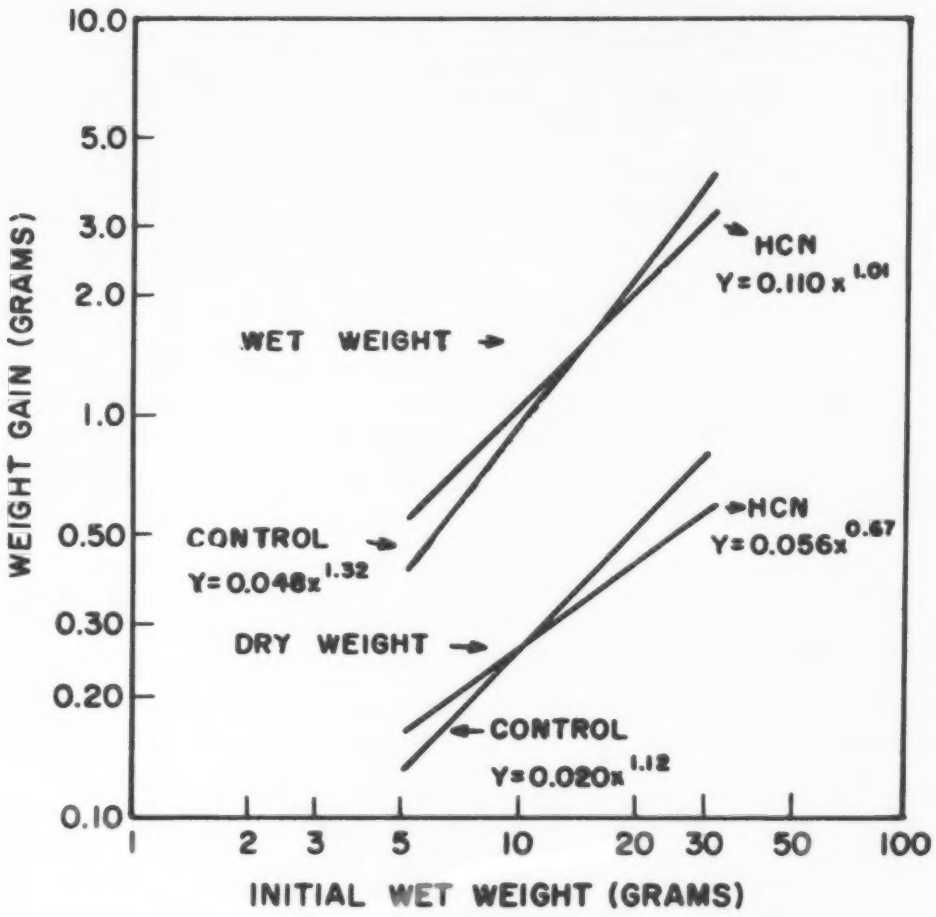


Figure 4-10. Regression lines relating wet- and dry-weight gains of control and HCN-exposed juvenile rainbow trout to their initial wet weights ranging from 5 to 30 g while they were swimming against a current of 12.1 $cm.s^{-1}$ at 10°C. A statistical analysis determined that the slopes of the lines representing the control and cyanide-exposed fish were significantly different on both a wet- and dry-weight basis (from McCracken 1978 and McCracken and Leduc 1980).

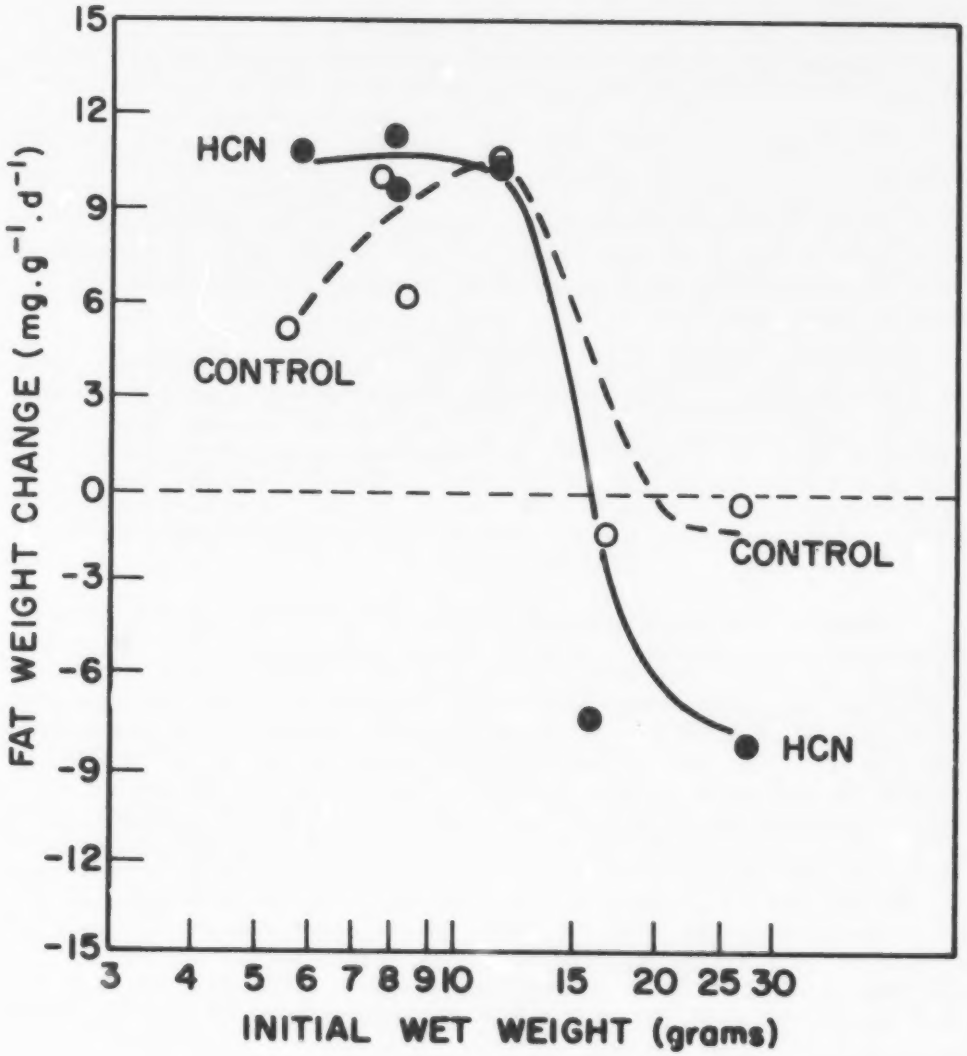


Figure 4-11. Relationship between fat weight changes of control and HCN-exposed juvenile rainbow trout and their initial wet weights (from McCracken 1978 and McCracken and Leduc 1980).

to a size of about 11 g whereas the bigger fish lost fat, just maintaining themselves near a zero rate of growth. The curve for cyanide-exposed trout reveals that the small fish had a faster buildup of fat content than the controls until a size of 11 g was reached; thereafter they lost substantially more than the controls. The curves intersect at an initial wet weight of 11 g where there is no difference in fat gain between control and exposed groups.

It should be noted that the changes in fat content closely approximate those changes based on wet and dry weight from the regression analysis (Figure 4-11). These observed weight changes should reflect either changes in metabolic pathways or changes in relative metabolic costs to small and large fish. It is not yet possible, however, to explain why small trout grew faster than the controls in the presence of cyanide.

Negilski (1973) also found that small salmonids may grow faster when exposed to low cyanide concentrations. Juvenile chinook salmon, Oncorhynchus tshawytscha, held in artificial streams (24 $cm.s^{-1}$) and feeding upon a diversified benthic community, were exposed to cyanide concentrations of 10 and 20 $\mu g.L^{-1}$ HCN for several weeks. In two experiments at 10 $\mu g.L^{-1}$ the growth rates, final biomass and production of salmon were higher than in control groups whereas at 20 $\mu g.L^{-1}$ HCN some reduction was noticeable.

Speyer (1975) investigated the effects of cyanide-arsenic mixtures upon fish growth. Rainbow trout averaging 20 to 28 g wet weight were exposed over a 21-day period at 11°C. The adverse affect of arsenic alone on wet weight (i.e. not a cyanide-arsenic mixture) is apparent only at 6.0 $mg.L^{-1}$ As (Figure 4-12); however, for dry weight changes the results are different since some reduction of growth is noticeable at 3.0 $mg.L^{-1}$ As. Cyanide alone arrested dry weight gain whereas in combination with 6.0 $mg.L^{-1}$ As it produced dry weight loss. These results suggest that the effect of the toxicants was to increase the water content of the fish while simultaneously promoting dry weight loss. The effects of arsenic and cyanide were even more drastic on fat gain. Compared with the controls which gained about 20% in fat, rainbow trout exposed to 1.0, 2.0 and 3.0 $mg.L^{-1}$ As gained only 7 to 10%; at 6.0 $mg.L^{-1}$ they lost over 5% of their fat reserves. The combinations 3.0 and 6.0 $mg.L^{-1}$ As with 20 $\mu g.L^{-1}$ HCN produced losses of fat of about 20%.

4.2.3.4 Swimming Ability

Cyanide has a strong, immediate and long-lasting inhibitory effect on the swimming ability of fish. Neil (1957) and Broderius (1970) have observed that maximum inhibition (50 to 90%) was achieved within 24 hours of exposure. Various studies summarized in Figure 4-13 illustrate the great sensitivity of fish to chronic poisoning at concentrations below 100 $\mu g.L^{-1}$ HCN. The test conditions followed in these

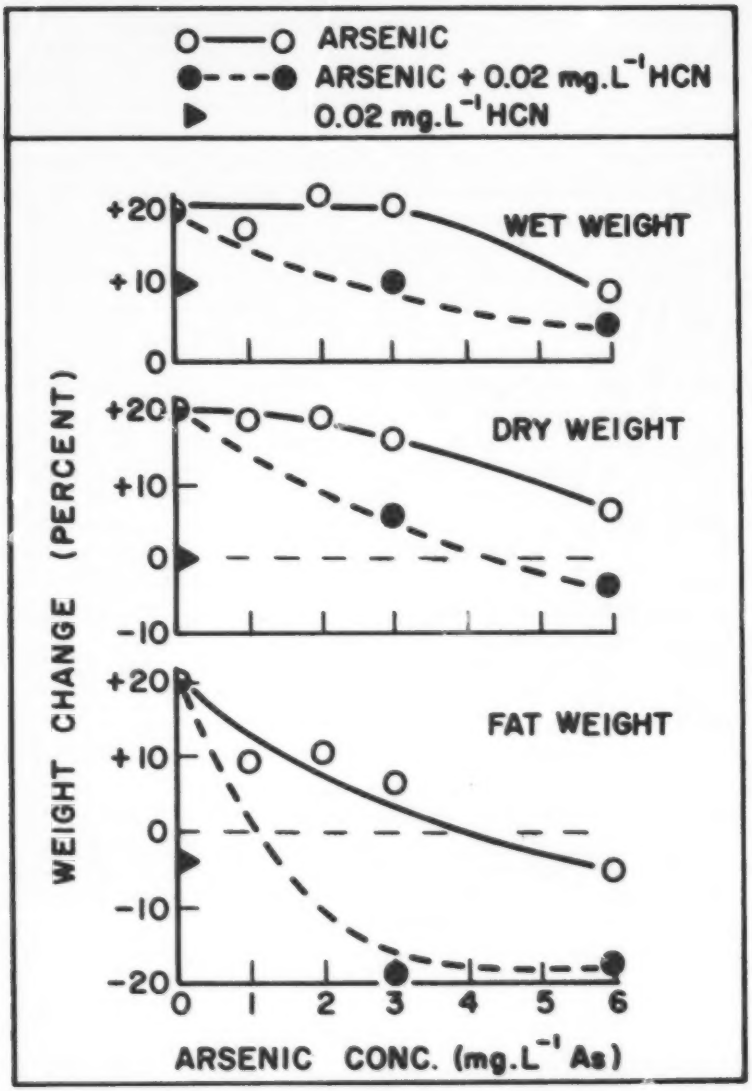


Figure 4-12. Effects of arsenic and HCN, singly or in combination, on wet-, dry-, and fat-weight gains of rainbow trout (adapted from Speyer 1975).

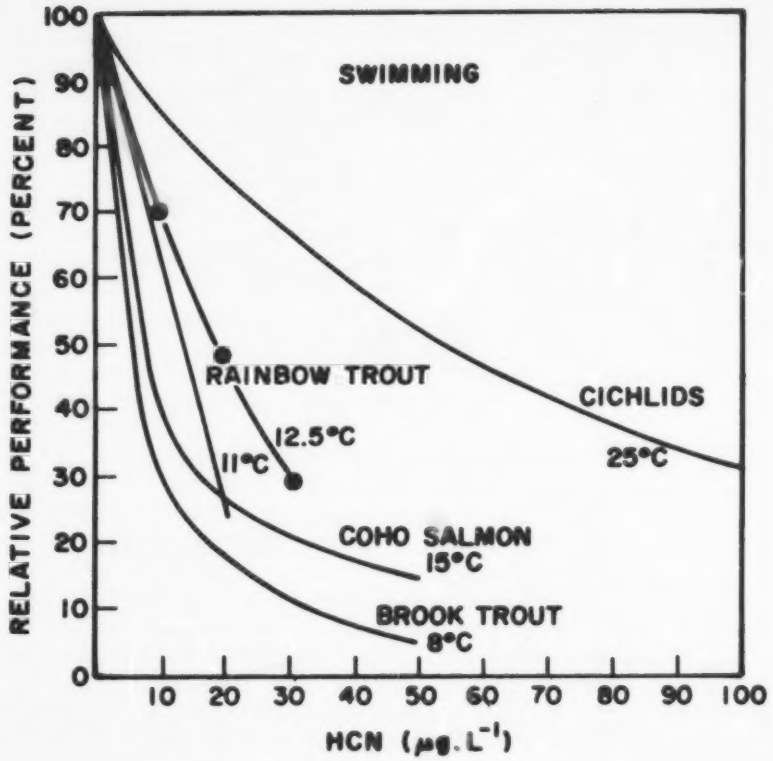


Figure 4-13. Effects of cyanide on the swimming endurance of various species of fish which had been exposed to sublethal levels of HCN for various periods (see text for discussions; see Table 4-6 for test conditions).

studies and presented in Table 4-6 varied considerably and may explain the apparent differences between the cichlids and the salmonids. As noted earlier for growth, the difference in swimming ability cannot be explained as simply reflecting specific differences in cyanide tolerance. The lower temperatures at which the salmonids were exposed during cyanide poisoning probably account for much of the apparent greater sensitivity of the salmonids.

Although the test conditions were far from being similar, there is an apparent relation between the degree of swimming inhibition caused by cyanide and temperature, the effect being more pronounced at lower temperatures. Kovacs (1979) and Kovacs and Leduc (1982b) have clearly demonstrated this phenomenon after testing the swimming activity of rainbow trout at the end of 20-day growth experiments in the presence of cyanide. The swimming tests were carried out in a Blazka type swimming chamber (Kruzynski 1972) in the absence of the toxicant. The duration of swimming at 53 $cm.s^{-1}$ was measured and the results expressed as the mean distance travelled (Figure 4-14). Cyanide reduced the swimming ability at all the concentrations tested. The effect increased markedly with decreasing temperature. For example, at 45 $\mu g.L^{-1}$ HCN and 18°C, swimming performance was reduced by about 26% but at 30 $\mu g.L^{-1}$ and 12°C this reduction was 71%. At 15 $\mu g.L^{-1}$ and 6°C the trout suffered a 90% reduction of swimming stamina.

The effects of cyanide on swimming are immediate and may last long after the fish have been returned to clean water. Even though Broderius (1970) has shown an immediate improvement of swimming after return of the fish to cyanide-free water, full recovery of chronically exposed salmonids was not complete 15 to 20 days after their removal from cyanide-containing water (Neil 1957; Broderius 1970).

These results are indicative of serious and perhaps permanent metabolic impairment caused by cyanide. However, since the inhibitory action of cyanide on cytochrome oxidase is reversible, one would expect recovery, unless pathways other than the cytochrome chain have been inhibited.

4.2.3.5 Respiration

Dixon and Leduc (1981) measured the resting metabolic rate of rainbow trout at 12.5°C after exposure to cyanide. After two 18-day growth experiments in which groups of rainbow trout had been exposed to 0, 10, 20 and 30 $\mu g.L^{-1}$ HCN, individual trout were placed in black tube flow-through respirometers and the oxygen consumption was measured for 6 consecutive days. The general pattern of the results is shown in Figure 4-15. The metabolic rate of the controls progressively dropped, probably as a result of quietening and food deprivation. The cyanide-exposed fish, however, initially elevated their respiration rate before stabilizing it at a higher level than that of the controls. We may

Table 4-6. Test conditions in different studies carried out to measure the effects of HCN on the swimming ability of fish.

Species tested	Period of exposure to cyanide (d)	Swimming speed ($cm.s^{-1}$)	Temp. (°C)	Reference
Salvelinus fontinalis	29	55.8 α	8.8	Neil (1957)
Cichlasoma bimaculatum	36	11.0 β	25.0	Leduc (1966)
Oncorhynchus kisutch	8	48.8 α	15.0	Broderius (1970)
Salmo gairdneri	20	53 β	11.0	Speyer (1975)
Salmo gairdneri	20	53 β	12.5	Kovacs (1979) and Kovacs and Leduc (1982b)

α Cyanide present in swimming chamber at the same concentration(s) as during the previous exposure periods.

β No cyanide for swimming test.

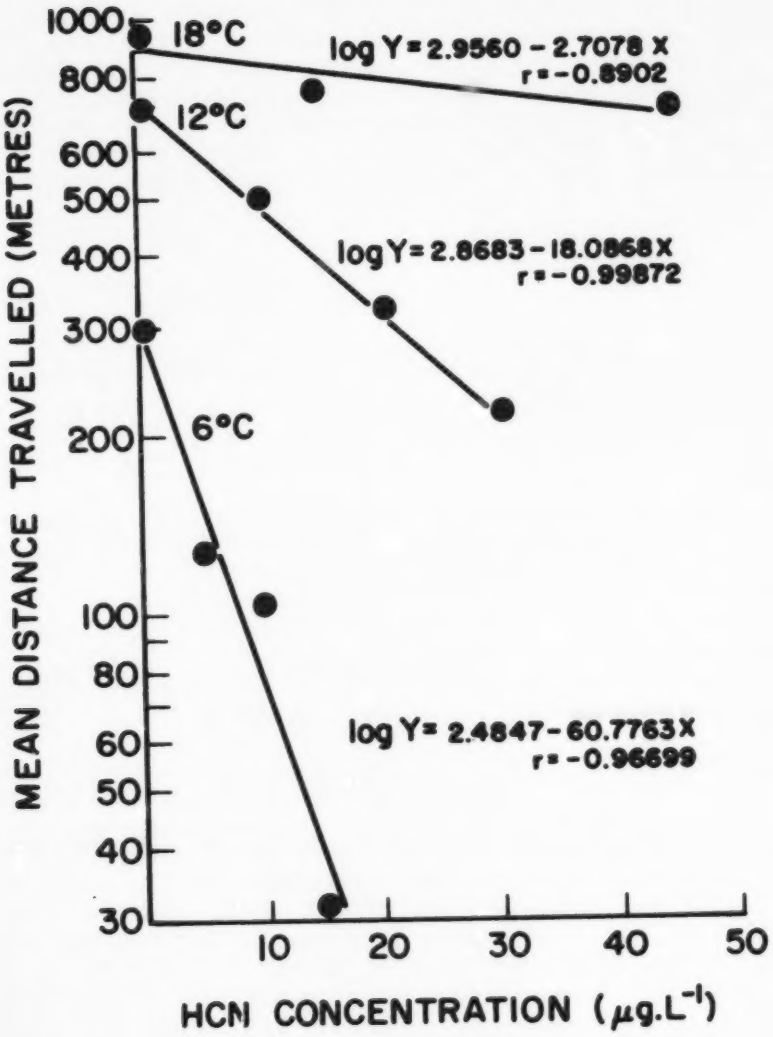


Figure 4-14. Relationship between mean distance travelled by juvenile rainbow trout and the concentrations of HCN to which they were exposed for 20 days at various temperatures (from Kovacs 1979 and Kovacs and Leduc 1982b).

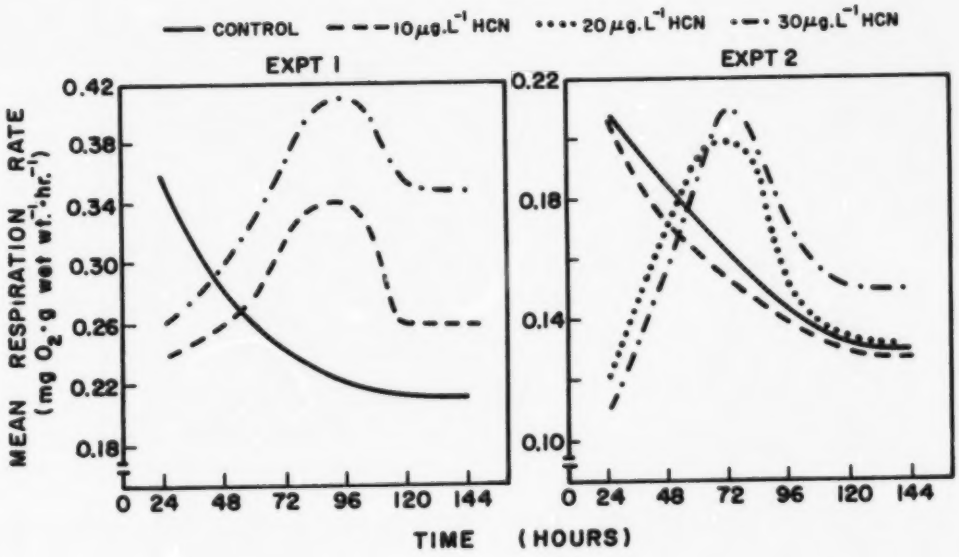


Figure 4-15. Changes in oxygen consumption of juvenile rainbow trout after exposure to various concentrations of HCN during 18-d growth experiments at 12.5°C. Each curve represents the values determined from 5 fish (from Dixon 1975 and Dixon and Leduc 1981).

speculate that the initial rise reflects a surge of oxidation of accumulated reduced metabolites when the fish were returned to clean water after 18 days of cyanide exposure. This stabilized higher metabolic rate may also be indicative of permanent damage that would reduce the metabolic efficiency of the fish or impose an extra metabolic load, both conditions resulting in an increased oxygen consumption. This increased cost of operation may reduce the scope of activity (Fry 1947) and may explain the very slow recovery of swimming performance of brook trout and coho salmon after exposure to chronic cyanide poisoning (Neil 1957; Broderius 1970). One must examine some basic physiological functions before attempting any explanation of this increased metabolic rate.

4.2.3.6 Osmo- and Ionoregulation

Although cyanide pollution may occur in coastal marine waters (cf. Côté 1976) there are no known studies of chronic toxicity of cyanide in seawater. The only available information regarding the influence of salinity on cyanide toxicity pertains to lethal levels (Broderius 1973) (see Section 4.2.2).

Osmoregulation accounts for an important fraction of the basic metabolic rate of freshwater fish since they have to continually excrete an excess of water brought in by the osmotic gradient; this work also increases with activity. This phenomenon has been well demonstrated by Rao (1968) who has calculated that osmoregulation may account for up to 20% of the active metabolic rate of rainbow trout. Ionoregulation is also a critical function which enables the fish to maintain the proper ionic strength in its tissues. The studies of osmo- and ionoregulation, in relation to the action of toxicants, are of double interest. They may provide highly informative clues as to the overall response of fish to a toxicant while in freshwater and/or when migrating to sea.

Along these lines, Leduc and Chan (1975) exposed rainbow trout to cyanide (10, 15, 21, 28 and 37 $\mu g.L^{-1}$ HCN) in renewed freshwater (10°C) for 28 days, then transferred them to artificial seawater ($18.9^{o}/oo$) containing no cyanide, before finally returning them to cyanide-free freshwater. Cyanide affected both osmo- and ionoregulation in saltwater and in freshwater. Figure 4-16a shows that after 260 hours in saltwater the plasma chloride and plasma concentration were higher in cyanide-exposed fish, whereas upon return to freshwater (Figure 4-16b) the reverse occurred, indicating loss of chloride and higher water content. In another test, it was shown that cyanide had an immediate effect on osmo- and ionoregulation when saltwater-adapted trout were transferred into freshwater-cyanide tanks (Figure 4-16c). These changes may look small and of questionable significance but Wood and Randall (1973) have demonstrated that changes as small as 1% correspond to a marked increase in urine flow, which is maintained by glomerular filtration concomitant with high energy expenditures. It therefore appears that although the effect of cyanide on the water content of the

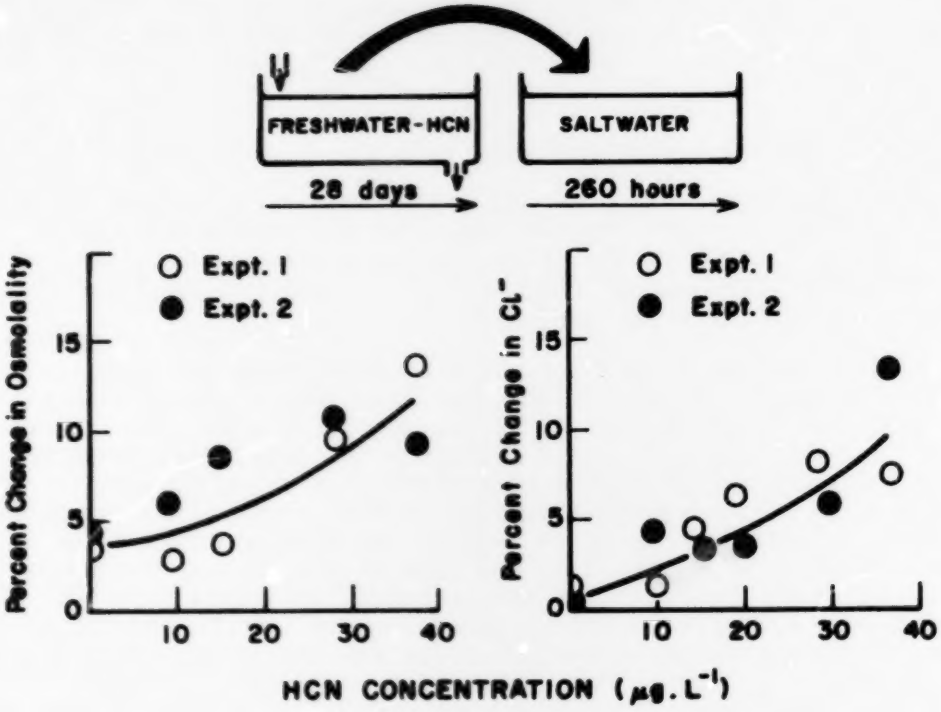


Figure 4-16a. Effect of HCN on osmoregulation by rainbow trout. Relationships between the changes of plasma composition that occurred during a 260-h salinity (18.9°/oo) tolerance test, and the HCN concentrations to which juvenile rainbow trout had been exposed for 28 days in flow-through aquaria at 10°C (from Leduc and Chan 1975).

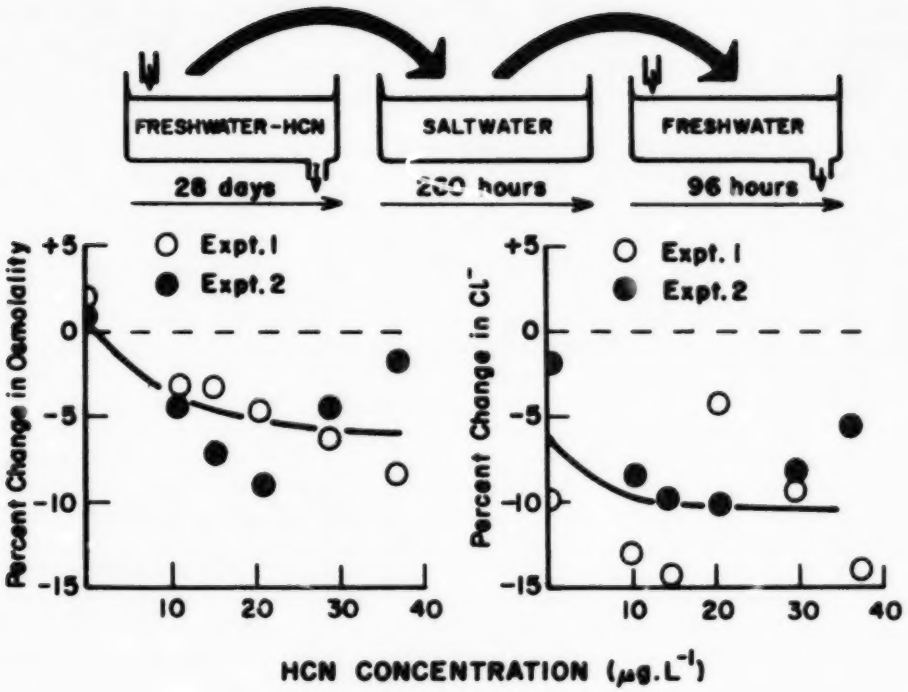


Figure 4-16b. Effect of HCN on osmoregulation by rainbow trout. Relationships between the changes of plasma composition that occurred during freshwater exposure following transfer of the fish from saltwater and the HCN concentrations to which juvenile rainbow trout had been exposed for 28 days in flow-through aquaria at 10°C (from Leduc and Chan 1975).

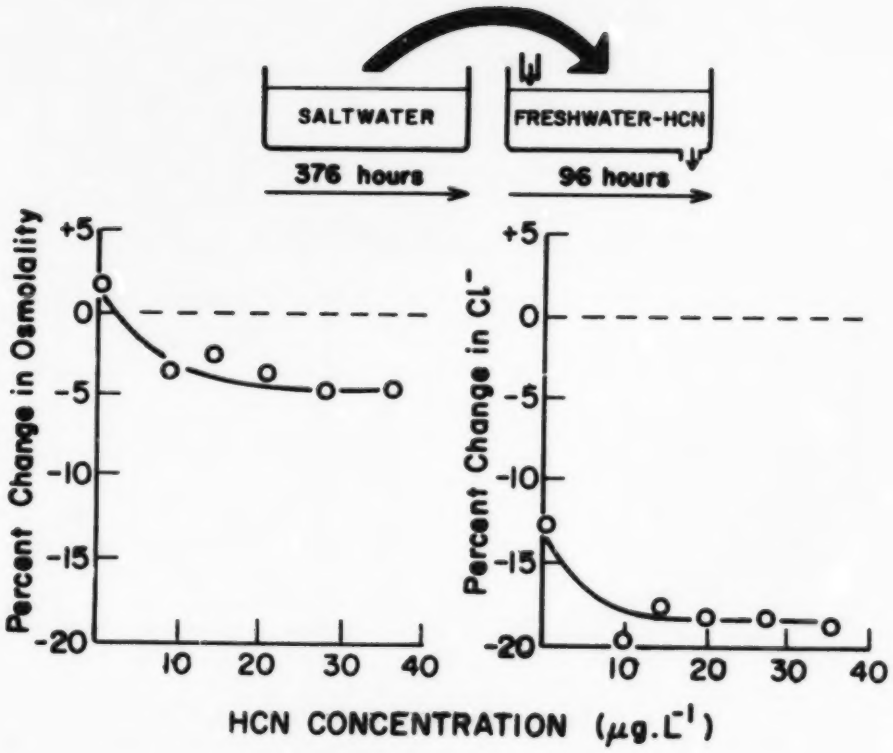


Figure 4-16c. Effect of HCN on osmoregulation by rainbow trout. Relationships between the changes of plasma composition that occurred during a 4-day freshwater-cyanide exposure following transfer from saltwater (18.9°/oo), and the HCN concentrations to which juvenile rainbow trout were exposed in fresh water flow-through aquaria at 10°C (from Leduc and Chan 1975).

fish is subtle, it may be indicative of serious physiological impairment.

4.2.3.7 Histopathological Effects

Not only did cichlids grown at 10 and 90 $\mu g.L^{-1}$ HCN have a markedly reduced swimming performance, but some showed serious body injuries at the end of the tests. Scales were falling off, and short handling with a dip net was sufficient to severely damage the fin rays. Some also showed swelling of the body and extensive subcutaneous hemorrhaging. After the tests all fish were returned to clean water (no current) for observation and some died within 24 hours (Leduc 1966).

Dixon (1975) and Dixon and Leduc (1981) reported that a 9-day exposure of rainbow trout to 10 $\mu g.L^{-1}$ HCN was sufficient to induce extensive necrobiosis (the degenerative process leading to cell death) in the liver; gill tissue from the same fish showed no apparent cyanide-induced histopathological damage. The examination of liver tissue from rainbow trout held at 10, 20 and 30 $\mu g.L^{-1}$ HCN for 9 and 18 days revealed different degrees of necrobiosis; the effect was well established after 9 days of cyanide exposure but showed no further intensification after an additional 9 days. At 10 $\mu g.L^{-1}$ cyanide the rainbow trout liver showed pycnosis, a condition involving contraction of the nucleus and condensation of the chromatin into one or more heavily stained clumps. At 20 $\mu g.L^{-1}$ disorganization was evidenced by a breakdown in the chord-like arrangement of the hepatocytes. In addition, cytolysis, which is manifested by an increase in cytoplasmic viscosity followed by swelling and bursting of the cytoplasmic membrane, occurred together with karyolysis, a condition involving a loss of stainability by the nucleus and its chromatin. The hepatic tissues taken from fish held at 30 $\mu g.L^{-1}$ exhibited an extensive breakdown in cellular organization as well as cytolysis and karyolysis.

While the symptoms of necrobiosis described above for each cyanide concentration were those which predominated at that concentration, the extent of tissue damage increased with the concentration of cyanide to which the fish had been exposed. One of the most striking phenomena evident in the histological results is the hepatic damage observed in the fish exposed to 10 $\mu g.L^{-1}$ HCN. While this group of fish showed no poisoning symptoms involving their general behavior, mean wet weight gain or respiration, there was extensive liver damage.

4.2.4 CHRONIC TOXICITY TO INVERTEBRATES

Studies concerning the long-term chronic toxicity of free cyanide to invertebrate populations are extremely limited in number. Oseid and Smith (1979) exposed Asellus communis and Gammarus pseudolimnaeus populations alone and together to various concentrations of HCN over periods of 98-115 days. For Asellus, when exposed alone, the highest concentration with any survival and reproduction was 317

$\mu g.L^{-1}$ HCN, although the highest no-effect level was in the range 29-40 $\mu g.L^{-1}$ HCN. Gammarus was less tolerant. The highest concentrations at which only a fraction of the population could survive and reproduce were 52 and 16 $\mu g.L^{-1}$ HCN respectively.

In a mixed community, Asellus was about as resistant as when tested alone whereas Gammarus was more sensitive. It appeared that the concentration of cyanide present could determine which species would dominate the other. The more aggressive Gammarus almost completely eliminated the Asellus from the controls and low treatment groups. However, Oseid and Smith (1979) speculated that at higher concentrations the competitive advantage would probably shift to Asellus, because of its much higher resistance to HCN.

4.3 OTHER RELATED COMPOUNDS

Although some simple cyanide-containing compounds have been identified in effluents, detailed studies on their effects on aquatic organisms have not been conducted. Some metallocyanide complexes undergo photolysis and release cyanate to the environment (Dauchy et al. 1980). Table 4-7 summarizes the acute toxicities of cyanate, thiocyanate and mercuric thiocyanate to some freshwater and marine organisms. Concentrations of mercuric thiocyanate in industrial effluents have not been reported in the general scientific literature. Cyanogen chloride is expected to occur in effluents which have been improperly treated. Concentrations of a cyanogen chloride of approximately 40 $\mu g.L^{-1}$ as total cyanide are known to be toxic to fish (Allen et al. 1948).

4.4 METALLO- AND ORGANOCYANIDES

Certain cyanides have been treated as entities capable of liberating free cyanide. Several metallocyanides and organocyanides have been identified thus far as having at least some toxicological aspects of free cyanide (Doudoroff et al. 1966; Broderius 1973; Daugherty and Garrett 1951; Henderson et al. 1961; Renn 1955; Kobayashi and Mori 1973). As a first approximation, therefore, the toxicity of cyanohydrins (e.g. lactonitrile) and metallocyanides in general may be estimated from the concentration of HCN detectable in the water at a given time.

4.4.1 METALLOCYANIDES

The cyanide ion is a strong nucleophile and will therefore form complexes with a number of heavy metals (see Chapter 2.0). The formation of a complex occurs when a dissolved metal salt such as $AgNO_3$ or $NiSO_4$ is mixed with a solution of simple cyanide. The complex is more or less soluble and may precipitate. It was thought that simple solutions of cyanide could be detoxified by treatment with a heavy metal salt. Milne (1950) first proposed this approach, whereby the cyanide

Table 4-7. Short-term toxicity of certain cyanide-containing compounds.

Cyanide salt	LC_{50}	Species	Aqueous medium	Temperature (°C)	Reference
Thiocyanate	87 $mg.L^{-1}$ as CNS^- (96-hour exposure)	mosquitofish (*Gambusia affinis*)	freshwater	20	Wallen *et al.* (1957)
Cyanate	18 $mg.L^{-1}$ as CNO^- (48-hour exposure)	water flea (*Daphnia magna*)	freshwater	-	Dauchy *et al.* (1980)
Mercuric thiocyanate	0.120 $mg.L^{-1}$ (96-hour exposure)	grass shrimp (*Palaemonetes pugio*)	marine	22	Curtis *et al.* (1979)
Mercuric thiocyanate	0.180 $mg.L^{-1}$ (96-hour exposure)	fathead minnows (*Pimephales promelas*)	freshwater	22	Curtis *et al.* (1979)

ion would be bound by nickel, rendering it nontoxic. However, in dilute solution the metallocyanide complex $M(CN)_x^{-w}$ will dissolve and form a dissociation equilibrium releasing a free cyanide ion:

$$M(CN)_x^{-w} \rightleftarrows M^{x-w} + xCN^- \qquad (4\text{-}12)$$

where M is generally a transition metal such as iron, nickel, cobalt, copper, zinc etc; x is the number of cyanide groups or ligands and w the oxidation state. It is evident that in a solution containing a dissolved metallocyanide complex two forms of cyanide coexist: the metallocyanide ion and the free cyanide ion. Although the metallocyanide ion may be toxic in itself, the toxic action is largely controlled by the amount of free cyanide that reacts with H^+ to form hydrocyanic acid, the most toxic species.

$$M(CN)_x^{-w} \rightleftarrows xCN^- + xH^+ \rightleftarrows xHCN \qquad (4\text{-}13)$$

Disregarding the inherent toxicity of the metallocyanide ion for the moment, it is evident that the toxicity of the solution will depend upon the stability of the complex and the available hydrogen ions or the pH. The most common metal cyanide complexes likely to occur in concentrated cyanide wastewaters (Section 2.5; Table 2-2) demonstrate the following order of stability: $Zn(CN)_4^{-2} < Cd(CN)_4^{-2} < Ag(CN)_2^{-1} < Ni(CN)_4^{-2} < Cu(CN)_3^{-2} < Hg(CN)_4^{-2} < Fe(CN)_6^{-4} < Fe(CN)_6^{-3}$.

Smith (1976) has calculated the amount of free cyanide in equilibrium with different concentrations of various metallocyanide complexes (Table 4-8). The examination of this table suggests two groups of complexes with regard to free cyanide. The complexes of mercury, silver, copper and ferri-ferro cyanide would appear to release only minor amounts of CN^- via dissociation, with concentrations of free cyanide all below 0.006 $mg.L^{-1}$ HCN. On the other hand, the complexes of nickel, cadmium and zinc potentially generate high, acutely toxic levels of free cyanide by dissociation.

However, there is reason to believe that Smith's (1976) calculations are not completely defendable in terms of amounts of free HCN in equilibrium with the various metallocyanide complexes and can, therefore, be misleading as to the toxic effect. Doudoroff *et al.* (1966), using a gas stripping sampling method followed by a gas chromatography determination, measured the actual amounts of free cyanide (HCN) in solutions of single cyanide salts as well as in solutions of metallocyanide complexes of nickel, copper, zinc, cadmium and silver at various pH's. The acute toxicity of these solutions was simultaneously measured by monitoring the median survival time (MST) of bluegills (*Lepomis macrochirus*). This way, by comparing the MST and the free cyanide levels in simple cyanide solutions (NaCN) and in complex cyanide solutions, it was possible to identify the active component(s) responsible for mortality. The toxicity of metallocyanide complexes was attributable almost solely to the concentration of free cyanide in equilibrium with the complex.

Table 4-8. Concentrations of free cyanide released at various concentrations of complex cyanides according to Smith (1976)and Doudoroff et al. (1966).

	Concentrations of free cyanide released at given concentrations of complex cyanides ($mg.L^{-1}$)				
Complex	($1\ mg.L^{-1}$)	($10\ mg.L^{-1}$)	($100\ mg.L^{-1}$)	($1{,}000\ mg.L^{-1}$)	($100{,}000\ mg.L^{-1}$)
$(Hg(CN)_4)^{-2}$	0.00002	0.00003	0.000045	0.00007	0.00018
$(Ag(CN)_2)^{-1}$	0.00009	0.0002 (0.2 pH 7.5)$^{\alpha}$	0.0004	0.0009	0.0041
$(Cu(CN)_3)^{-2}$	0.0003	0.0054 (1.20 pH 7.2)$^{\alpha}$	0.00097	0.0017	0.0054
$(Fe(CN)_6)^{-3}$	0.0002	0.0032	0.0004	0.0006	0.0012
$(Fe(CN)_6)^{-4}$	0.0012	0.0016	0.0022	0.0031	0.0061
$(Ni(CN)_4)^{-2}$	0.135	0.215	0.340 (0.46)$^{\alpha}$	0.539	1.324
$(Cd(CN)_4)^{-2}$	**	2.30	3.64	5.77	14.49
$(Zn(CN)_4)^{-2}$	**	2.20	3.59	5.68	14.28

** Calculations indicate that at this dilution, the two complexes are essentially completely ionized.

$^{\alpha}$ Measured values obtained from Doudoroff et al. (1966).

The levels of free HCN measured in complex cyanide solutions by Doudoroff et al. (1966) may be compared to those calculated by Smith (1976) (Table 4-8). The discrepancies between the calculated and measured free HCN levels can be very important. A difference of 25% in the case of the nickel complex is not significant since both concentrations (0.46 and 0.34 $mg.L^{-1}$) are acutely toxic. The differences are however, much more critical for the copper and silver complexes since Smith's calculation underestimates the lethal level for fish, when in fact much higher toxic levels were measured by Doudoroff et al. (1966). Moreover, had the tests performed by Doudoroff et al. (1966) been carried out at pH 7.0 rather than at 7.2 and 7.5, much higher levels of free HCN would have been measured. For example, at pH 7.0 a nickel-cyanide complex has a 96-h LC_{50} value of 1.4 $mg.L^{-1}$ as total cyanide whereas at pH 7.5 the 96-h LC_{50} is 21 $mg.L^{-1}$ (Doudoroff 1956). Consider the overall dissociation equilibria:

$$Ni(CN)_4^{-2} \rightleftarrows Ni^{+2} + 4CN^- + 4H^+ \rightleftarrows 4HCN \qquad (4\text{-}14)$$

For an increase of 0.3 units from pH 7.0 the hydrogen ion concentration decreases by a factor of 2 or $\frac{10^{-7}}{2}$ since

$$pH = \log \frac{1}{10^{-7}/2}$$

$$= 7 \log 10 + \log 2$$
$$= 7.3$$

Such discrepancies between measured and calculated values of individual chemical species are unfortunately not limited to metallocyanide equilibria. With the general trend toward computational methods in aquatic toxicology, one should be aware that stability constants may lack the precision and accuracy required for toxicity evaluation. In his first studies of the metallocyanide complexes, Doudoroff (1956) recognized the disparity between his toxicity data and the published stability constants, and subsequently suggested that the published values were erroneous. A more pertinent set of constants developed since that time are included in Table 2-2 (see Section 2.5).

An inherent toxicity has also been associated with the metallic component of tetracyanonickelate II $[Ni(CN)_4]^{-2}$, dicyanoargentate I, $[Ag(CN)_2]^{-1}$, and dicyanocuprate I, $[Cu(CN)_2]^{-1}$. Structure-activity relationships for these complexes are discussed in Section 2.5 and will not be reiterated here. In the case of the nickel-cyanide complex, an extrapolation of the existing data shows that the time-mortality curve is more closely aligned with that of nickel than that of free cyanide. Specifically, the slope of the curve for the nickel-

cyanide complex at approximately 96 hours of exposure may be estimated as follows:

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calculated 96-h LC_{50} = 950 $\mu g.L^{-1}$ as total cyanide
calculated 168-h LC_{50} = 650 $\mu g.L^{-1}$ as total cyanide
for fathead minnow (Pimephales promelas)
at 20°C (Doudoroff 1976):

$$\text{estimated slope} = \frac{650 - 950}{168 - 96} = -4.2$$

The slope of a time-mortality curve for fish exposed to nickel alone is:

calculated time to 50% mortality at 41 $mg.L^{-1}$ Ni = 53 h
calculated time to 50% mortality at 23 $mg.L^{-1}$ Ni = 125 h
for rainbow trout (Salmo gairdneri) at
12°C (Anderson et al. 1979):

$$\text{estimated slope} = \frac{53 - 125}{41 - 23} = -4.0, \text{ at approximately 96 h of exposure}$$

These slope estimates may be interpreted to mean that the mortality of fish exposed to either nickel alone or the nickel-cyanide complex continues beyond 96 hours for what is, as yet, an undefined time period. In contrast, the mortality rate for fish exposed to free cyanide apparently ceases after 96 hours (Cardwell et al. 1976). The actual values (i.e. -4.0, -4.2) cannot be compared statistically; however, similar modes of action might be proposed if the toxicity curves were parallel. Conceivably, coordination bonding between tissue sites and the metal on the nickel-cyanide complex (see Section 2.5) may be intimately associated with a common mode of action. Considering Doudoroff's (1976) calculations, one may suggest that tetracyanonickelate II at concentrations of approximately 1.0 $mg.L^{-1}$ as total cyanide is toxic to fish.

The other toxic metallocyanides (i.e. silver and copper cyanides) may be perceived differently. As these complexes are readily dissociable, and as the toxicity of the metal is much greater than that of the metallocyanide, detrimental effects can be estimated from total metal concentrations (e.g. toxic threshold concentration 10 - 40 $\mu g.L^{-1}$ total dissolved copper in freshwater or marine environments (Spear and Pierce 1979); and, 0.10 - 0.25 $\mu g.L^{-1}$ total silver in freshwaters and 30 $\mu g.L^{-1}$ total silver in marine waters (Thurston et al. 1979)). No scientific criteria based on the toxicity of mercury- and cobalt-cyanide complexes exist at the present time. Also, inherent toxicity associated with chromium-, zinc-, and lead-cyanide complexes is unlikely due to chemical treatment of industrial wastes (see Section 2.5).

4.4.2 ORGANOCYANIDES

Organocyanides differ widely in their toxicity to fish. The cyanohydrins (e.g. lactonitrile) are perhaps the most important group because of their hydrolysis in aqueous media releasing free HCN. The toxicity is expressed in terms of total cyanide and is comparable to that of free cyanide. Henderson et al. (1961) determined that the 96-h LC_{50} of lactonitrile in renewed water to the fathead minnow was about 270 $\mu g.L^{-1}$ as total cyanide. Generally short-term lethal concentrations for freshwater fishes range from 190 to 500 $\mu g.L^{-1}$ (Renn 1955; Henderson et al. 1961).

Other organocyanides such as acetonitrile, benzonitrile, adiponitrile, malononitrile and acrylonitrile are considerably less toxic than lactonitrile and unlike lactonitrile, do not release free cyanide in aqueous media (Henderson et al. 1961). Table 4-9 summarizes the acute toxicity of those compounds to various species of fish. Several attempts have been made to elucidate a lethal threshold for acrylonitrile. However, lethality has been shown to occur after long-term exposures, with no clear indication of a threshold (Table 4-9). Sublethal chronic toxicity studies using nitriles have not been conducted.

4.5 MULTIPLE TOXICITY

Lethal and sublethal effects of binary mixtures of cyanide and chromium, zinc or ammonia to the fathead minnow and to rainbow trout have been studied by Broderius and Smith (1979). Their study shows that neither the response addition nor the concentration addition models (Anderson and Weber 1977) could be applied to predict the acute dose-mortality responses for the mixtures tested. For the mixture Cr-HCN, about 20% mortality was predicted, but the mixture in fact killed about 50%. Similarly the Zn-HCN and NH_3-HCN mixtures killed about 50% compared with a prediction of < 1% mortality. The toxicant concentration ratios used to determine equal proportions of toxic units were 266, 20.9 and 12.2 for the Cr-HCN, Zn-HCN and NH_3-HCN mixtures respectively. The type of multiple toxicity observed for fish exposed to lethal concentrations of mixtures did not occur at sublethal levels in growth studies. The only mixture producing a greater reduction in dry-weight gain than predicted was the NH_3-HCN combination. For the Cr-HCN and Zn-HCN mixtures the effect was equivalent to that determined from the toxicant tested alone. These results show different physiological interactions at high and low concentrations of the same toxicants.

In another study, Speyer (1975) (see Section 4.2.3.3) subjected rainbow trout to binary mixtures of As-HCN for 21 days and measured growth in the form of wet-, dry- and fat-weight changes. Here the model of response addition (Finney 1971) seemed to explain the interaction of the arsenic and cyanide acting independently but producing a similar response.

Table 4-9. Acute toxicity of some organocyanides to freshwater fish, expressed as $mg.L^{-1}$ of the compound.

Compound	LC_{50}	Species	Reference
acetonitrile (CH_3CN)	1000[α]	fathead minnow	Henderson et al. (1961)
	1850[α]	bluegill	Henderson et al. (1961)
benzonitrile (C_6H_5CN)	135[α]	fathead minnow	Henderson et al. (1961)
	78[α]	bluegill	Henderson et al. (1961)
adiponitrile ($CN(CH_2)_4CN$)	1250[α]	fathead minnow	Henderson et al. (1961)
	720[α]	bluegill	Henderson et al. (1961)
malononitrile ($CNCH_2CN$)	1.63[α]	rainbow trout	Abram and Wilson (1979)
acrylonitrile (CH_2CHCN)	18.1[α]	fathead minnow	Henderson et al. (1961)
	11.8[α]	bluegill	Henderson et al. (1961)
	33[β]	rainbow trout	Jackson and Brown (1970)
	1.6[γ]	rainbow trout	Jackson and Brown (1970)

[α] 96-hour exposure.

[β] 48-hour exposure

[γ] 100-day exposure.

5.0 OVERALL SIGNIFICANCE OF LABORATORY STUDIES

The bulk of the literature pertaining to cyanide aquatic toxicology is derived from laboratory studies, carried out on fish that have never been previously exposed to cyanide and which have been held at fixed concentrations (usually HCN) for the duration of the tests. Although the test conditions are unrealistic compared with the natural environment, such studies have been necessary in order to understand the basic mechanisms of cyanide intoxication. From such studies, cyanide has been shown to markedly reduce the performance of several physiological functions of fish. However it is difficult to arrive at a comprehensive significant judgment as to the toxicity of HCN.

For example, acclimation of fish through continuous exposure to low levels of cyanide may enhance their resistance to potentially lethal concentrations (cf. Doudoroff 1976). In addition, low pulsating levels of cyanide may enable the exposed fish to recover and/or initiate detoxification mechanisms. Unless questions of adaptive resistance to lethal levels of cyanide and/or the long-term effects of discontinuous sublethal concentrations are verified, at least on fish and other aquatic organisms in the laboratory, it becomes very difficult to assess the effects of natural or anthropogenic pulses of cyanide when coupled with the variation of other important environmental parameters such as oxygen, pH and temperature.

In an attempt to arrive at an overall evaluation of the chronic toxicity of cyanide, the approach taken by Warren *et al.* (1973) was followed by Leduc (1977). It consists of drawing, on the same graph, experimentally obtained relative performance curves plotted against HCN concentrations while giving a value of 100 to the controls. Leduc (1977) plotted performance curves (Figure 5-1) and drew a "Relative Performance Index" curve (heavy trait) as a single generalized response curve to HCN. The relative performance index curve suggests a 50% reduction of total performance of cold-water laboratory fish held at 10-13°C and continuously exposed to 10 $\mu g.L^{-1}$ HCN for 20-30 days. Even though fish in some laboratory studies have been found to survive at 30 $\mu g.L^{-1}$ HCN, Figure 5-1 suggests that the different physiological requirements necessary to survive in nature could not be met. Although no single concentration limit of free cyanide can be accepted as a universally applied water quality objective (Doudoroff 1980), Figure 5-1 suggests that cyanide in the concentration range of 3-5 $\mu g.L^{-1}$ HCN would result in relatively minimal impairment of the "Relative Performance Index" of freshwater fish, as calculated from laboratory studies. Such a concentration range would appear to be an appropriate water quality objective for freshwater fish in water of 10-13°C; such a range coincides with those proposed by Doudoroff *et al.* (1979), Doudoroff (1980) and EPA (1980).

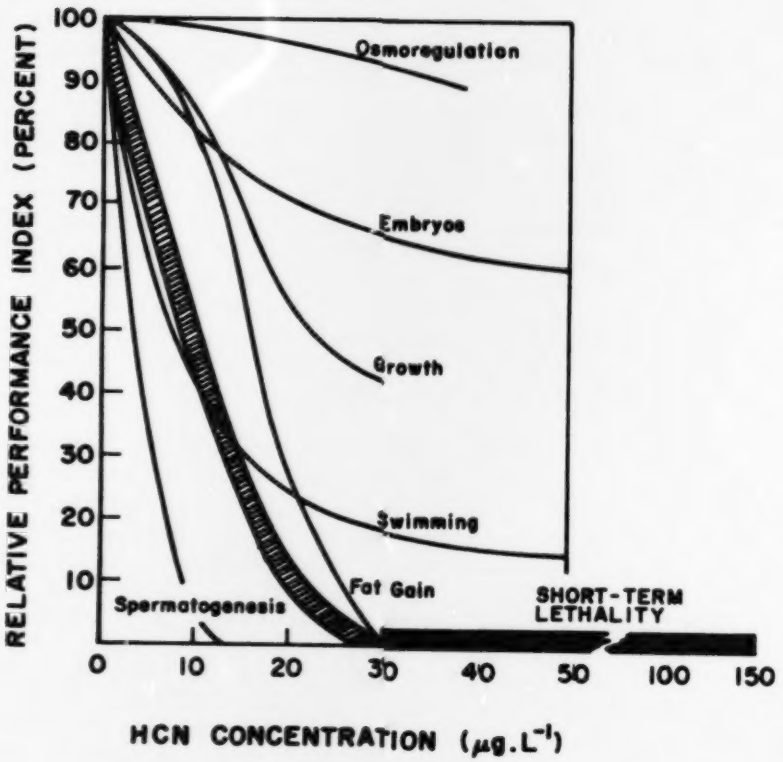

Figure 5-1. A generalized summation of the sublethal effects of HCN upon fish (from Leduc 1977).

APPENDIX I CALCULATION OF ENERGY BUDGET FOR HCN-EXPOSED FISH

Several assumptions have been made in order to calculate the energy budgets from changes in wet weight and fat content which were measured by McCracken (1978) and McCracken and Leduc (1980). The water content of a typical fish is 75-80% (Phillips 1969). Therefore, wet weights were multiplied by 0.25 to estimate dry body weight. Dry weights so calculated may not accurately represent fat contents because fish tend to assimilate fats in place of water and vice versa (Phillips 1969). Therefore, the percent fat which may be included in the estimated dry-weight values was ignored and constitutes an error in the calculations. Measured fat contents were taken to be accurate. The diet composition was 49.5% protein, 9.7% fat, and 28.3% carbohydrate on a dry-weight basis (McCracken 1978 and McCracken and Leduc 1980). Caloric equivalents were assumed to be 4.8 $cal.g^{-1}$ for protein, 9.5 $cal.g^{-1}$ for fat, and 5.4 $cal.g^{-1}$ for carbohydrate. The average value, 5.1 $cal.g^{-1}$, was applied to the protein-plus-carbohydrate compartment. Food assimilation efficiencies, corrected for nitrogen loss, were assumed to be 90% protein, 85% fat and 40% carbohydrate (Phillips 1969). The heat increment was calculated as 0.23 of energy supplied in the 1.5% diet. The metabolic requirement was taken from Brett (1965) at comparable swimming speed and size of fish.

APPENDIX II

GLOSSARY

alevin - developmental stage occurring in fish species lacking a larval period, lasting until scalation is complete or until spines become ossified (occurs mainly in salmonids)

ataxia - lack of muscular coordination as a result of any of several nervous system disorders

atresia - involution of a part (as an ovarian follicle)

blastulation - formation of a blastula from a solid ball of cleaving cells

concentration addition - the constituents of a pollutant mixture have qualitatively similar toxic effects and these effects combine additively, although the concentration of one or another required to produce a given effect may be quite different
- if the response is equivalent to that expected for the algebraic summation of dosages, then the effect is termed strict addition
- if the response is less than that predicted for strict addition, the effect is termed infra-additive
- if the response is greater than that predicted for strict addition, the effect is termed supra-additive

einstein - a unit of light energy used in photochemistry equal to Avogadro's number times the energy of one photon of light of the frequency in question

eleutheroembryo - developmental stage commencing with hatching and lasting until most or all of the yolk is digested and the fish begin to feed externally or until feeding on the ovarian secretion is terminated by parturition

hyperthyroidism - excessive activity of the thyroid gland resulting in an increased metabolic rate, enlargement of the thyroid gland, rapid heart rate and high blood pressure

hypothyroidism - deficient activity of the thyroid gland resulting in a lowered metabolic rate and general loss of vigor

photoaquation - formation of a complex that contains water by replacement of other coordinated groups in the complex, caused by the action of radiant energy

photolysis - chemical decomposition by the action of radiant energy

prokaryote - any cell containing a primitive nucleus, where the deoxyribonucleic acid-containing region lacks a limiting membrane e.g. bacteria and blue-green algae

protopterygiolarval phase - encompasses the interval between the transition to exogenous feeding and the commencement of differentiation of the embryonic median fin fold
- does not occur in salmonids

quantum yield - for a photochemical reaction, the number of moles of a stated reactant disappearing or the number of moles of a stated product produced, per einstein of light of the stated wavelength absorbed

response addition - the constituents of a pollutant mixture, although acting on different systems (i.e. independently) may contribute to a common response, if their respective concentrations are equal to or greater than the threshold computed from quantal response curves for single toxicants

toxic unit - calculated by dividing the actual concentration of each toxicant in a mixture by its lethal threshold concentration
- if the sum of the concentrations of the individual toxicants expressed in toxic units is > 1.0, the mixture is predicted to be lethal

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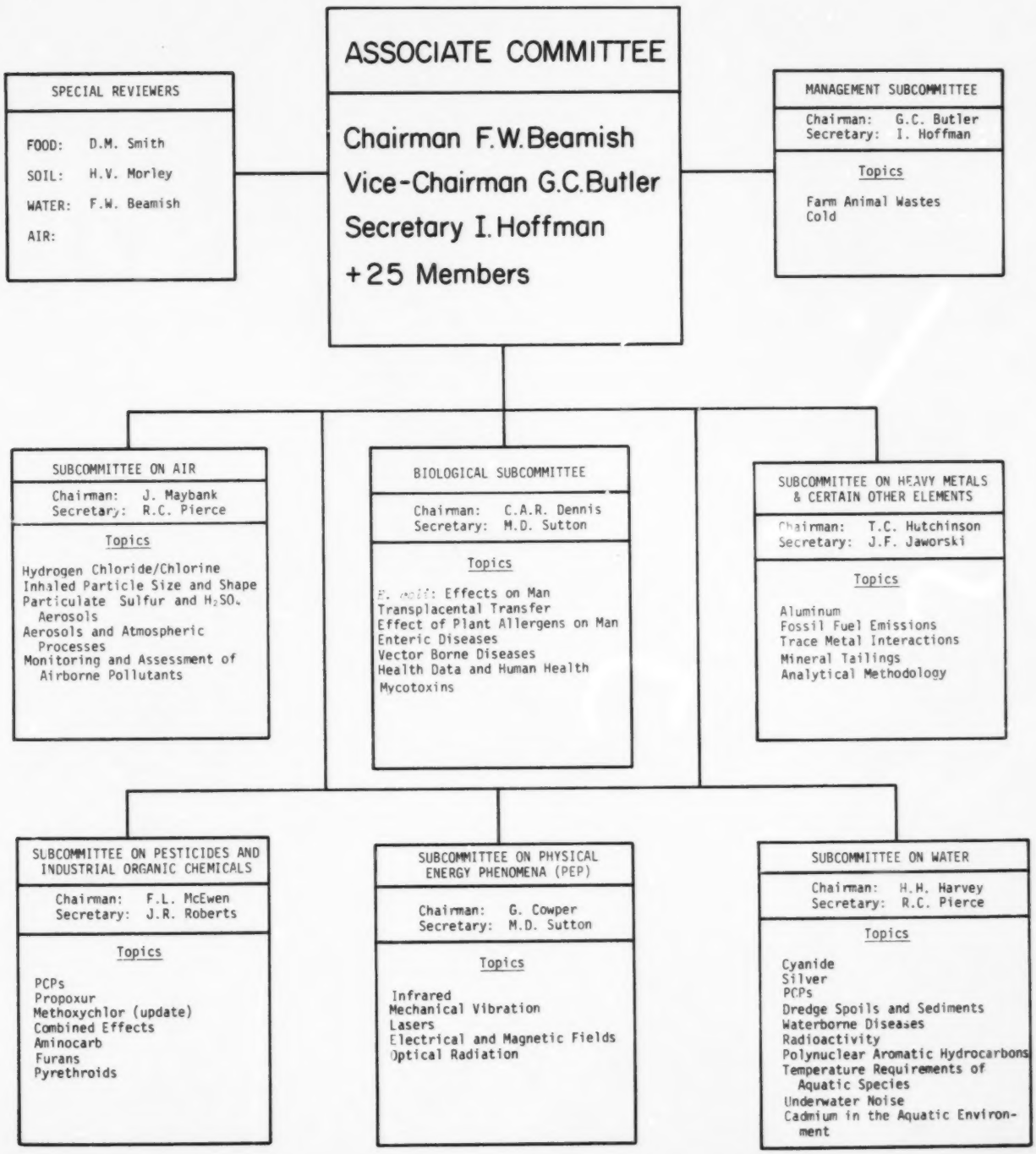
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